VOLUME LXXVI NUMBER 3

THE
BOTANICAL GAZETTE

November 1923

XEROPHYTISM AND COMPARATIVE LEAF ANATOMY IN RELATION TO TRANSPIRING POWER

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(WITH PLATES XX–XXIII)

Since STAHL (7) pointed out many years ago the possibility of using paper impregnated with cobalt chloride to demonstrate transpiration, numerous investigators have endeavored to perfect a reliable quantitative method based upon STAHL's suggestions. All of this work has been based upon the well known property of cobalt chloride appearing blue when dry, and red or pink (or quite colorless) when hydrated, if placed in dilute form in specially prepared paper slips. The method involves the application of strips of paper containing cobalt chloride, or "hygrometric paper," to the surface of the leaf and observing the rate of color change produced by the water vapor emanating from the leaf and absorbed by the cobalt paper.

The earlier work of this nature was of qualitative value only, but nevertheless of importance in the development of a striking colorometric method for the demonstration of the loss of water in the invisible vapor form from plants. American physiologists and ecologists have taken the lead in the later development of the method, particularly with the attempt to perfect a useful quantitative instrument based upon the principles involved. LIVINGSTON (4) conceived the idea of a standardized evaporating surface to

which the power to give off water to the hygrometric paper was compared directly with the similar power of the leaf surface. Later work by the same investigator and others, including BAKKE (1, 2), LIVINGSTON and TRELEASE (6), and LIVINGSTON and SHREVE (5), led to the perfection of a standardized cobalt chloride paper suitable for careful work and to the development of a method which might be applied to plants growing in their natural environment. The methods used in making the field observations and in preparing the data described in this paper were essentially those described by LIVINGSTON and SHREVE.

Method

Standardized cobalt paper slips with standardized permanent color standards may now be purchased under the name of "tripartite slips." They consist of two permanent standard colors (blue) mounted upon opposite sides of the hygrometric paper, the whole composite slip being about 12 mm. long by 4 mm. wide, the three segments being bound together by narrow bands of nonabsorbent silk adhesive. Each of the standard colors is blue, one being a little less intense than is the color of the thoroughly desiccated cobalt chloride paper mounted in the center, while the other is much less intense but still clearly and perceptibly blue. The two permanent color standards are prepared by precipitating Prussian blue in filter paper of the same kind used in preparing the cobalt chloride paper. The precipitation is affected by bringing ferric chloride and potassium ferrocyanide together in the paper. A proper variation of certain processes in the development of the paper renders readily possible the preparation of the two permanent color standards, one relatively dark and the other very light blue. Such colored papers, if properly prepared, do not suffer any color alterations "when exposed to air and sunshine for several days, and they do not alter their color with changes in hygroscopic moisture, such as are obtained when they are placed over a standardized evaporating surface or upon a leaf."

Each of the composite paper slips (tripartite) when finished by this method consists of a small thin square of hygrometric paper, mounted between two similar squares of permanent blue standard

paper, the whole bound together by black non-absorbent adhesive tissue. "Each of the three paper surfaces visible to the observer when the strip is applied to the leaf is about 4 mm. square and is surrounded by a black border about 1 mm. wide." By this arrangement the two standard colors are automatically placed in position underneath a glass clip whenever the sensitive paper is applied to any surface to be tested. The standard colors are so closely placed by the side of the hygrometric paper as to render comparison of tints readily and quickly possible. The standard color papers also absorb moisture, but the colors are not thereby modified, while the cobalt chloride paper changes from deep blue to almost pure white.

The composite slips are dried after use by heat or by placing them in a desiccator. They must be kept in some form of desiccator until they are to be applied to the surface to be tested. It was found convenient for field use to prepare several dozen small desiccators, each to hold a single composite slip, which could be carried about in a pack, and which were otherwise readily adapted to field use. Small glass vials (10 cc.) fitted with rubber stoppers were used for this purpose. Each vial was nearly filled with anhydrous phosphoric acid which was covered with a circle of fine gauze, the paper slip being placed on the gauze and the vial kept tightly closed with a rubber stopper. It was found that the slips remained dry indefinitely under these conditions. After the slips had been used it was found very convenient to take them from the desiccators, and by means of forceps hold them for a moment near a warm electric iron until the hygrometric paper became dried out to a point where it was dark blue, usually darker in color than the dark standard. After drying, the slips were returned to the desiccators where they were kept until ready for further use. Each slip was numbered to correspond with the desiccator in which it was kept.

A further requirement for the successful application of the hygrometric paper method is in the nature of a standard evaporating surface, with which the behavior of the sensitive test papers may be compared. LIVINGSTON and his students (**4**, **5**, **6**) have also perfected this feature of the method to such a degree as to remove one of the main difficulties in the operation of the method as a

whole. It is now readily possible to derive a permanent standardization for the paper slips in the laboratory. "Each composite slip is to be thoroughly tested in the laboratory under known temperature conditions, and from the average time period derived from a number of such tests, a correction coefficient is calculated for each temperature at which field observations are made." Records are kept by slip numbers as the tests are made, and finally the average time period for color change of each particular slip at 20° C. is found by calculation, and is expressed as a single number characteristic for that slip. Knowing this number and also the air temperature at which any field test is made, the time period for this slip at the latter temperature (on the standard evaporating surface) may readily be found by employing published tables of aqueous vapor pressures. In short, the method involves merely the use of hygrometric slips which have been standardized over a standard evaporating surface, the values being computed on the basis of 20° C., and which may be applied to the leaf surface under temperature and time conditions determined for each test. The readings are then computed on the basis of the standard values for the various slips.

The field kit used in the investigations reported in this paper consisted of a box containing fifty of the small desiccators described, each with its numbered tripartite slip, half a dozen clamps for holding the slips in position on the leaf, a small pair of forceps for handling the slips, a stop watch, and a good thermometer. Some strips of woolen fabric were included as packing to prevent the breakage of any of the apparatus in the kit in moving about from station to station.

The necessary technique involved in making the field tests is very simple. A plant is selected for a series of tests, and a thermometer is set up in such manner as to record the temperature in the immediate vicinity of the leaves to be tested. The thermometer is shaded and the tests are also made in the shade, in the shadow cast by the body of the operator if there is not some other source of shade. The stop watch and the clamps are placed in readiness. A desiccator is taken from the box and the rubber stopper withdrawn by holding the desiccator in the left hand and pulling the stopper with the thumb and forefinger of the same hand. Then

with the forceps in the right hand, the composite slip is quickly withdrawn and the stopper replaced. With the left hand the leaf clamp (which had previously been clamped on the leaf) is carefully released, and the composite slip quickly placed upon the surface of the leaf and wholly covered by the clear glass plate of the clamp. With a little practice this operation may be performed very quickly, so that no perceptible change occurs in the hygrometric paper during the transfer from desiccator to leaf. When the slip is in position and held there firmly by the clamp, the stop watch is set, and then the central square on the composite slip (the hygrometric paper) is very carefully watched as its deep blue color gradually fades until it just matches the darker of the two permanent standards. When that point is reached the watch is started, as the observer follows the change in the hygrometric paper. When the color developed by hydration of the cobalt chloride in the hygrometric paper matches the lighter permanent color standard, the test is regarded as having been completed and the watch is stopped.

"Since the two color standards are permanent in color, they furnish two fixed points in the series of color intensities that are presented as the test paper alters from intense blue to pink, and the test now becomes simply to determine the length of time elapsing between the occurrence of a fixed strong intensity of color and that of an equally fixed weak intensity." The number of the slip used, the temperature near the leaf, and the time for the color change are recorded in the notebook under the appropriate designation of species, habitat, etc. If material for anatomical study is to be collected, it should be taken at the conclusion of the tests or before leaving for some other species or station.

It is a pleasure to acknowledge the efficient assistance rendered by one of my graduate students, Miss LEONA GILMORE, in connection with the histological and microphotographic methods involved in this paper. The plates are wholly her work.

Transpiring power and habitat differentiation

The index of transpiring power is regarded as the ability or the measure of the power of a leaf to supply moisture vapor to the surrounding air. As determined by means of cobalt paper, the transpiring power represents the ability of a leaf to give off water

vapor, and is measured in terms of the similar power possessed by a standard evaporating surface at the same temperature. Values less than unity are for leaves with lower power of giving off water than is possessed by the standard evaporation surface. Those greater than unity are for leaves with greater power of giving off water than the standard surface at the same temperature. In a certain sense this power may be regarded as the resistance offered by leaves to the diffusion of water vapor from their surfaces. The factors controlling this water loss are wholly internal, hence transpiring power must not be confused with transpiration as the latter phenomenon is usually understood.

BAKKE (**2**) suggested that a knowledge of the transpiring power of plants might be of considerable importance in connection with studies of drought resistance in cultivated crops, and in connection with the relative xerophytism exhibited by different species. It seemed desirable to follow this suggestion in connection with a study of certain plant associations of the Rocky Mountain region, in the hope that a factor might be discovered which would be helpful in the differentiation and classification of the various vegetation units involved in that investigation. Accordingly, considerable time was utilized during the summer of 1921 in testing out the method with numerous species of a number of the plant associations in the Pike's Peak region. It was also desired to learn whether there is any fairly reliable correlation between transpiring power as determined by this method, and such features of plant development as leaf anatomy and other phenomena which are commonly included in the estimation of the relative xerophytism of different species. The method has already been employed by BAKKE in a study of certain field crop and native plants of southwestern United States.

Forty representative species were selected from the many available forms to be found in the various plant communities, from the shortgrass mesas and plateaux east of Colorado Springs and up through the foothill bushlands to the subalpine forest associations, and the alpine meadows above an altitude of 12,000 feet. From this list a number of species have been selected for anatomical study and for a comparison of transpiring power as observed in the

different habitats. A list of the principal species included in this study, together with the respective indices of transpiring power, are recorded in table I. The values indicated represent the average transpiring power of all determinations made on upper and lower surfaces of each species respectively, beginning with the most xerophytic forms and closing with the more mesophytic. The values have been calculated from published tables and other data given in literature cited at the end of this paper. The original tables of data secured in the field and the numerous calculations necessary to arrive at the final indices have been omitted for the sake of conserving space.

If we are to accept the suggestion made by BAKKE that all plants exhibiting a foliar transpiring power of less than 0.30 may be regarded as xerophytes, while those with indices above 0.70 may be considered mesophytes, then these data show a number of evident discrepancies. Certainly practically all forms of *Quercus Gambellii* and *Cercocarpus parvifolius* must be considered xerophilous, although the indices of transpiring power for these species are considerably above the limit set by a conspicuous xerophyte (*Chrysopsis villosa*) of the shortgrass plains of early summer, while still others farther down the list would doubtless be classified as xerophytes by most ecologists, on a basis of habitat distribution. The same conclusion would probably be reached after a consideration of the comparative leaf anatomy of the forms in question. Only seven species in this list would fall within the limits of xerophytism, as defined by BAKKE, while a considerably longer list (fourteen) would lie beyond the limit set for mesophytes. This is in spite of the fact that the greater number of the species are characteristic of sites which are decidedly xerophilous.

In this list the writer would draw the line between the xerophilous and mesophilous species at 0.9088, which is the index of transpiring power for *Pentstemon unilateralis*, a common form of the foothills. Certainly all of the other species in the list, *Geranium Richardsonii* (shade form), *Melilotus alba*, *Mertensia ciliata*, *Lactuca scariola*, *Streptopus amplexicaulis*, *Poa pratensis*, and *Chamaenerion angustifolium* are clearly defined mesophytes in distribution.

TABLE I

INDEX OF FOLIAR TRANSPIRING POWER FOR PLANTS GROWING IN NATURAL HABITATS, JULY AND AUGUST 1921

Species	Total transpiring power
1. Chrysopsis villosa	0.1370
2. Rhus trilobata (sun form)	0.1023
3. Clematis ligusticifolia	0.1808
4. Allionia linearis	0.1810
5. Actinella acaulis	0.2160
6. Allionia hirsuta	0.2702
7. Quercus Gambellii	0.2706
8. Astragalus hypoglottis	0.3100
9. Petalostemon candidus	0.3472
10. Cercocarpus parvifolius (sun form)	0.3523
11. Quercus Gambellii (shade form)	0.4181
12. Pulsatilla hirsutissima	0.4474
13. Rhus trilobata (shade form)	0.4694
14. Cleome serrulata	0.4825
15. Geranium Richardsonii (sun form)	0.4825
16. Gaura parviflora	0.5005
17. Senecio oblanceolatus	0.5176
18. Senecio cernuus	0.5567
19. Cercocarpus parvifolius (shade form)	0.5586
20. Andropogon furcatus	0.5920
21. Psoralea tenuiflora	0.6415
22. Populus tremuloides	0.6415
23. Populus angustifolia	0.6416
24. Thermopsis arenosa (sun form)	0.6521
25. Salix reticulata	0.6961
26. Pentstemon glaucus	0.7482
27. Thermopsis arenosa (part shade)	0.7791
28. Bistorta bistortoides	0.7845
29. Eriogonum umbellatum	0.8200
30. Euphorbia cuphosperma	0.8497
31. Pentstemon unilateralis	0.9088
32. Melilotus alba	1.0375
33. Geranium Richardsonii (shade form)	1.0651
34. Mertensia ciliata (deep shade)	1.5061
35. Lactuca scariola	1.5838
36. Phleum pratense	1.5721
37. Streptopus amplexicaulis (deep shade)	1.8207
38. Poa pratensis (deep shade)	1.8057
39. Chamaenerion angustifolium	1.8986

Since a number of the species immediately preceding these mesophytes are characteristic of habitats not quite so mesophilous, it is well to designate them as xero-mesophytes. Such a list would include *Euphorbia cuphosperma*, *Eriogonum umbellatum*, *Bistorta bistortoides*, *Thermopsis arenosa* (part shade form), *Penstemon glaucus*, *Salix reticulata*, *Thermopsis arenosa* (sun form), *Populus angustifolia*, and *P. tremuloides*. Then would come another series of species with lower indices of transpiring power, which may be called meso-xerophytes. This list would include *Psoralea tenuiflora*, *Andropogon furcatus*, *Cercocarpus parvifolius* (shade form), *Pulsatilla hirsutissima*, *Senecio cernuus*, *S. oblanceolatus*, *Gaura parviflora*, *Geranium Richardsonii* (sun form), *Cleome serrulata*, *Rhus trilobata* (shade form), and *Quercus Gambellii* (shade form). Beginning with the next in the series, *Cercocarpus parvifolius* (sun form) with an index of transpiring power of 0.3523, the remaining species of the list are most certainly to be regarded as xerophytes. Such a classification composed of four groups would care fairly well for the species included in the list. Placement according to that series is in partial accord, at least, with the common distributional phenomena as exhibited by the various species under examination.

It is interesting to note, in passing, that all of the polydemics included in the list exhibit a fairly consistent correlation between transpiring power and the relative xerophytism of their several habitats. The sun forms of *Rhus*, *Quercus*, *Cercocarpus*, *Geranium*, and *Thermopsis* exhibit in each case a lower transpiring power than that obtained for the shade forms of the same species.

The relative position of the three xerophytic dominants in the foothill brushland communities (in which most of this work was done) as regards their relative distribution is not correlated with the total average transpiring power of the sun and shade forms of the three species. Thus such index for *Rhus trilobata* is 0.3158, that for *Quercus Gambellii* is 0.3443, and that for *Cercocarpus parvifolius* is 0.4554. This is in inverse relation to the relative xerophytism of the habitats of which the three species are characteristic, within the areas where these studies were made.

It would thus appear that there is not as great a degree of correlation as would be expected between the relative xerophytism of the habitats in question and the index of transpiring power of various species of plants more or less characteristic of the sites under investigation, and as determined by the method of hygrometric paper. This is particularly noticeable in connection with the dominants of the foothill chaparral association. In other words, it would seem from these data that the facts which we may secure by the careful application of this method may possibly be of some assistance in the final differentiation of habitats, and in the development of a natural classification of the plant communities of a given area, but it is also possible that grave errors may arise from the use of the method.

Transpiring power and leaf anatomy

Little if any work is yet available to indicate the relation of transpiring power to the finer features of leaf anatomy. BAKKE (1) pointed out in a general way that certain species with which he worked were "undoubtedly xerophytic in leaf structure," that certain others were "typically mesophytic," and that "no plants of extreme mesophytic leaf structure were included in the series." He did not, however, report any investigations conducted with particular reference to the discovery of the closer relations between transpiring power and leaf histology.

Mrs. CLEMENTS (3) has pointed out a number of striking relations between the physical factors of the habitat and leaf structure. The field work reported in this paper was done in the same general region where her investigations were conducted. While she made no attempt to use the hygrometric paper method as an aid, nevertheless some of her findings are of interest in connection with the present investigations. Mrs. CLEMENTS' researches covered a great many of the species of the Pike's Peak region, and were representative of a wide variety of habitats.

After many years of research, ecologists are now fairly well agreed as to the general features in the histology of leaves which may be regarded as reactions to the conditions of xerophytic and mesophytic sites. There is not so nearly uniform agreement, however,

as to the causes or factors involved in the production of the xerophytic and the mesophytic leaf. It is difficult to separate and segregate the particular anatomical features of the leaf due to light from those due to water, for instance. Nevertheless, we do have a fairly definite conception of the xerophytic and the mesophytic leaf as anatomically distinct types.

At this point the common histological features usually associated with the two types of leaves may be mentioned, since they may serve as standards to which the various forms later described may be compared.

The xerophytic leaf is relatively thick, and sometimes without chlorophyll at the center. The epidermis is sometimes very thick and waxy, resinous or heavily cutinized, and the stoma-bearing surface is frequently hairy, oftentimes in contrast with the smooth upper surface. The stomata are sometimes protected by being placed beneath overarching projections of the cuticle, or inclosed by deep depressions of the epidermis. The guard cell walls are commonly cutinized, although experimental evidence is sufficient to account for but a slight increase in such cutinization by reference to xerophytic conditions. The structural rigidity of the stomata is noteworthy. Xerophytic leaves commonly display prominent palisade, often in several very densely packed layers, extending from one epidermis to the other. The palisades in the lower part of the leaf are sometimes less prominently developed or at least less densely arranged than in the upper portion of the leaf. The maximum development of palisade is said to occur in the driest and most exposed sites, where the light intensity is high, where transpiration is strong, and where absorption is relatively low. On the other hand, the sponge, or lower chlorenchyma, is commonly poorly developed, or at least is comparatively densely packed, with a corresponding decrease in the amount of intercellular air space. The development of intercellular air spaces or lacunae is usually regarded as being reciprocally related to the development of palisade and sponge tissues. The xerophytic leaf also commonly shows considerable development of "water storage" cells, especially in the immediate vicinity of the vascular strands. Such are the cells frequently distributed in the form of a clearly differentiated

bundle sheath, in which the cells are wholly lacking in chloroplasts. Bands of such cells are also to be noted extending from the bundle sheath to either epidermis in many xerophytic leaves. The maximum development of water storage cells or tissue is often seen in succulent xerophytes, in which they take various forms. It has also been pointed out that xerophytic leaves are likely to reveal the presence of more internal glandular or secretory cells or reservoirs than is the case in mesophytes. The rôle of such structural features, however, can scarcely yet be indicated on a physiological or ecological basis. As a rule, also, the fibrovascular elements are more copiously developed in the xerophyte than in the mesophyte.

Of all the histological features of the leaf the chlorenchyma is probably the most plastic, or most readily modified by environmental variations, so that some of the commonest and most striking differences betwen xerophytic and mesophytic leaves are to be found in the relative development of palisade and sponge, and in the relative proportion of lacunar volume in the chlorenchyma as a whole. The mesophytic leaf tends to have less palisade or a less densely packed palisade than the xerophytic leaf, with the corresponding differences in lacunar development. The spongy mesophyll is likely to be much more prominent in the mesophyte, and, in short, the entire chlorenchyma of the mesophyte is likely to be much more open and also thinner as a whole than in the xerophyte. The entire leaf of the mesophytic species is usually thinner than the xerophytic leaf. This is notably the case when we compare sections of the leaves of mesophytic and xerophytic forms of the same species. The mesophytic leaf also exhibits much less modified epidermal and stomatal conditions. Nevertheless, mesophytes commonly show a fairly well developed cuticle, particularly in those species which are to be regarded as xero-mesophytes, or forms which are clearly intermediate between the typical forms of xerophytes and mesophytes.

It is natural to expect that measurable difference in transpiration and in the transpiring power of leaves with such strikingly different anatomy might be found by appropriate methods. Differences have been recorded concerning the relation of these conditions to transpiration in the ordinary sense. One of the objects of this

investigation was to determine whether there are any relations between the transpiring power of leaves and their histological organization that may be determined by the hygrometric paper method. Forty-five to fifty species were selected for such study. These were the same species (including a number of sun and shade forms) noted earlier in this paper, most of which are included in table I.

It has been pointed out that there is some degree of correlation, although uncertain in some cases, between the transpiring power of those species and forms and the relative xerophytism of the various sites. This is indicated in the relative position of the various plants in the table, which are arranged in the sequence of their transpiring power.

When leaf anatomy is examined in connection with the relative position of the various species in the table, some interesting facts are revealed. In the first place, those species with unmistakably strongly developed xerophytic leaf anatomy are found in the fore part of the table, that is, among those in which the indices of foliar transpiring power are relatively low. This is in accordance with what is probably to be expected, since this power is determined very largely by internal forces and conditions. External forces and conditions which operate more or less prominently upon transpiration are practically absent under the conditions of the tests made by the method used in this work. The very compactness of the chlorenchyma of the xerophyte presents a real obstacle in the way of the evaporation of water in the interior of the leaf, resulting thereby in a reduction of the index of transpiring power or the ability to give off moisture vapor. Thus, as BAKKE (1) has pointed out, the index of transpiring power is low for xerophytes and relatively high for mesophytes. An inspection of the list of species herewith presented bears out this relationship in a convincing manner. It will be agreed that such species as *Rhus trilobata*, *Allionia linearis*, *Quercus Gambellii*, and *Cercocarpus parvifolius*, for instance, under usual distributional relationships are to be considered as xerophytes. On the other hand, such species as *Chamaenerion angustifolium*, *Poa pratensis*, *Streptopus amplexicaulis*, and *Mertensia ciliata* are undoubtedly typical mesophytes.

These two series occupy positions toward the opposite extremes as indicated in the values of the transpiring power. The histological features of these species also show that the relative position of the two groups is reflected in the nature of the tissues involved. The degree of correlation, however, is not as high as an enthusiast for this method might desire. In *Rhus* (fig. 5) it will be observed that the palisade is composed of very long and densely arranged cells, constituting about two-thirds of the total volume of the chlorenchyma. There is also a very noticeable mixture (not shown in the figure) of cells which contain tannin, resin, etc., scattered through the mesophyll. *Allionia linearis* (fig. 6) and *A. hirsuta* (fig. 4) illustrate even a greater extreme of xerophytism in their leaf structure, since the entire mesophyll is very compact and the lower (usually the sponge) portion of that region is scarcely less open than the upper or palisade portion. Both of these species have very thick leaves, with some tendency to succulence, and both have a well developed cuticle, while *A. hirsuta* is further characterized by a roughly hirsute or pilose surface. In *Quercus Gambellii* (fig. 3) the structure is denser than in either *Allionia* or *Rhus*, and the cuticle is heavier, although the index would indicate that the oak is less xerophytic. In *Cercocarpus parvifolius* (fig. 23) the palisade is made up of smaller cells, very compactly arranged, while the sponge is relatively open, but the stoma-bearing surface is thickly clothed with long silky or canescent hairs, and there is a row of very large water-storage cells just beneath the upper epidermis. On the whole, the latter species appears to be more xerophytic in structure than the others, but it is noted that it occurs considerably lower in the list, that is, the index of transpiring power is higher than in *Rhus*, *Quercus*, or *Allionia*.

Turning now to the consideration of the most typical mesophytes of table I, we find the leaves of *Streptopus amplexicaulis* (fig. 27), *Mertensia ciliata* (fig. 28), and *Chamaenerion angustifolium* (fig. 32) exhibit the histological features of the mesophyte as it is usually conceived. The leaves are much thinner than those of the previously mentioned xerophytes, there is only slight cutinization of the outer epidermal cell walls, and the chlorenchyma is remarkably loose and open in each case. In *Streptopus* there is no typical

palisade at all, while in *Mertensia* and *Chamaenerion* there is a single plate of loosely grouped, broadly prolate palisades, and the sponge is extremely loose with very large lacunar volume. These species fall among the last six of the list; in other words, they are among those exhibiting the highest index of transpiring power, or among those whose internal resistance to water outgo is relatively slight. It may be noted that the transpiring power in each of these cases is above unity.

The two sets of species noted mark fairly well the typically xerophytic and the typically mesophytic extremes of the series. Between these extremes there are a number of other species whose leaves exhibit even a greater degree of xerophytism than any of the xerophytes mentioned so far. Among those which are certainly xerophytic, on an anatomical basis, are *Actinella acaulis*, *Astragalus hypoglottis*, *Pentstemon cendidus*, *Cleome serrulata*, *Geranium Richardsonii* (sun form), *Senecio oblanceolatus*, *Gaura parviflora*, *Thermopsis arenosa*, *Bistorta bistortoides*, and *Pentstemon unilateralis*. All of these would most certainly be classified as xerophilous by most histologists on the basis of leaf anatomy. The highest index of transpiring power for this list is 0.9088 for the last species.

In looking over the list, together with an examination of the figures, it will be noted that there are certain other species whose relative positions in the sequence of index values are not quite what would be expected from a study of the anatomy of the leaf. We should ordinarily regard *Clematis ligusticifolia* as a mesophyte. The plants of the species which were used in this study, however, were growing over an old fence in almost maximum light and in very dry soil; yet the leaf structure is without question mesophytic. Perhaps the value determined for these plants does not represent the true value for the species, errors being due possibly to stomatal behavior, possibly to incipient drying of the leaf, or to some other cause which was not detected at the time the field tests were made. There are no other striking abnormalities of this nature revealed by the indices and figures. There are, however, a few cases between the indices of 0.6415 (*Psoralea tenuiflora*) and 0.9088 (*Pentstemon unilateralis*) upon which ecologists would probably differ as to the

relative positions indicated by the transpiring power and leaf anatomy respectively. For instance, the leaf of *Pentstemon unilateralis* shows a more xerophytic structure than that of *Populus angustifolia*, yet the index for the latter is lower (0.6416) than that for *Pentstemon* (0.9088). Most ecologists would probably hold, on a basis of the usual criteria, that *Pentstemon unilateralis* is more xerophytic than *Populus angustifolia*, although the sequence of the indices of transpiring power is quite the reverse.

The indices of transpiring power for sun and shade forms show some interesting relations in connection with leaf histology. The sun form of *Rhus trilobata* has a transpiring power of 0.1623, while the shade form shows an index of 0.4694. The shade leaf is only slightly more than one-half as thick as the sun leaf, and with a single row of broad and loosely arranged palisades. The palisade region of the sun leaf is composed of relatively narrow and compactly arranged cells, in which there is an irregular and more or less alternate arrangement of two-rowed palisades with greatly elongated palisade cells, with a length approximating twice the length of the shorter cells. The sponge is about equally open in both types, and with about the same proportion of lacunar space.

The sun and shade forms of the leaf in *Quercus Gambellii* are also strikingly different, although the differences are of the same general types as in *Rhus*. The sun leaf shows three compact plates of palisade, and two to four less regular plates of vertically elongated sponge cells with considerable lacunar space. The shade leaf is about one-half as thick as the sun leaf, and there are only two layers of palisades, which are also less densely packed than in the sun leaf. The sponge tissue and lacunar volume are more typical of the mesophyte than is the case in the sun leaf. Both leaves are rather heavily cutinized, but there is little difference between the two in this regard.

The sun and shade forms of leaf in *Thermopsis arenosa* and *Geranium Richardsonii* reflect similar general relations between habitat and histology, but the correlation of anatomy and transpiring power is not wholly as one may surmise. The sun form of *Geranium* is undoubtedly more mesophytic than the corresponding form of *Thermopsis* from the standpoint of histological organization,

yet the transpiring power of the former is 0.4825, while that of the latter is 0.6521, a very considerable difference, but with the expected order reversed. The viscid glandular nature of the surface of *Geranium*, however, coupled with the presence of much internal resinous or gelatinous (probably water-retentive materials), might more than counterbalance the effect of the denser mesophyll and smooth surface in *Thermopsis*. Oddly it is noted that the normal, expected conditions prevail in the shade forms of these two species, but it should be pointed out that the internal water-holding features are less noticeable in the shade form of *Geranium*, and that the glandular epidermal condition is also greatly reduced.

The three alpine species, *Salix reticulata* (fig. 21), *Pentstemon glaucus* (fig. 22), and *Bistorta bistortoides* (fig. 26), present some interesting features. These are all common alpine forms in the Rocky Mountains. The field readings were made upon plants, and material was collected for the histological work at an altitude of 12,000 feet on a typical bright day in August. The willow was growing in one of the characteristic densely caespitose or carpet-like forms, the well known habit of the species, while *Pentstemon* and *Bistorta* were scattered in the usual manner over the alpine meadow.

The sequence of transpiring power for these species is *Salix* 0.6961, *Pentstemon* 0.7482, and *Bistorta* 0.7845. The leaf of the willow is considerably thinner than the leaves of either of the other two, and the three plates of palisades are very densely grouped, with slight intercellular passages, while a rather close sponge and well developed cuticle complete the prominent histological features.

The leaf of *Pentstemon* is nearly twice the thickness of the *Salix* leaf, but the palisade is less dense and the sponge is more open, and there is a very well developed cuticle over both upper and lower epidermis. There are stomata in both epidermises, however, while in the willow stomata are confined to the lower epidermis. In the case of *Bistorta* the differences are not striking; yet a thinner cuticle and a more open chlorenchyma throughout are important conditions to be considered in placing the species on a basis of increasing mesophytism.

This order checks fairly well with the anatomy of the leaves, *Salix* being unquestionably the least mesophytic on the histological basis. It should be noted, however, that the differences in transpiring power between these three species are rather slight, a still further aid in the development of the conclusion that the three are very closely related in the matter of environment. The three species are to be regarded as mesophytes with a very decided tendency toward the xerophytic. In a broad classification they would probably all be placed among xerophytes, as are most Arctic alpine species.

Conclusions

The degree of correlation between habitat xerophytism and leaf anatomy and transpiring power as recorded by this method is rather low and uncertain. Although the relations appear in a fairly typical form to be expected in the extremes in the series, yet the ecological rank of the dominants and other species in the xerophytic foothill chaparral association, as indicated in table I, is quite the reverse of that determined by the usual criteria. There are also numerous puzzling features associated with the relations between leaf anatomy and the index of foliar transpiring power; yet the data in that connection are in general less unsatisfactory than those dealing with habitat differentiation.

The writer is wholly unable to explain the discrepancies of major value and other less noticeable irregularities described on the basis of the data so far accumulated. Possibly this condition is what we should expect in those species which are more or less intermediate between typical xerophytes and mesophytes. It is possible that the hygrometric paper method is not sufficiently delicate or accurate to render absolutely reliable quantitative data sufficient to differentiate perfectly between the more nearly similar forms. It is also probable that certain physiological processes and conditions that the method cannot evaluate enter and modify the accuracy of the tests. I am of the opinion, for example, that the hygrometric paper method would be of much more value, and the facts derived from its use more dependable, if the question of stomata and stomatal activities were carefully included in the investigations. It is a familiar fact that stomatal behavior affects ordinary transpiration

in a number of remarkable ways, and we should expect that such behavior would also modify transpiring power in some significant manner or degree. This is a line of investigation that should be followed by students of transpiring power and of the cobalt paper method. At present I am inclined to believe that the indices of transpiring power as determined by this method cannot properly be interpreted in the absence of a knowledge of stomatal behavior in the species concerned.

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EXPLANATION OF PLATES XX–XXIII

PLATE XX

FIGS. 1–8.—Fig. 1, *Rhus trilobata* (shade), ×80; fig. 2, *Petalostemon candidus*, ×75; fig. 3, *Quercus Gambellii* (sun), ×80; fig. 4, *Allionia hirsuta*, ×40; fig. 5, *Rhus trilobata* (sun), ×80; fig. 6, *Allionia linearis*, ×48; fig. 7, *Quercus Gambellii* (shade), ×80; fig. 8, *Actinella acaulis*, ×42.

PLATE XXI

FIGS. 9–16.—Fig. 9, *Clematis ligusticifolia*, ×80; fig. 10, *Pulsatilla hirsutissima*, ×80; fig. 11, *Astragalus hypoglottis*, ×75; fig. 12, *Cleome serrulata*,

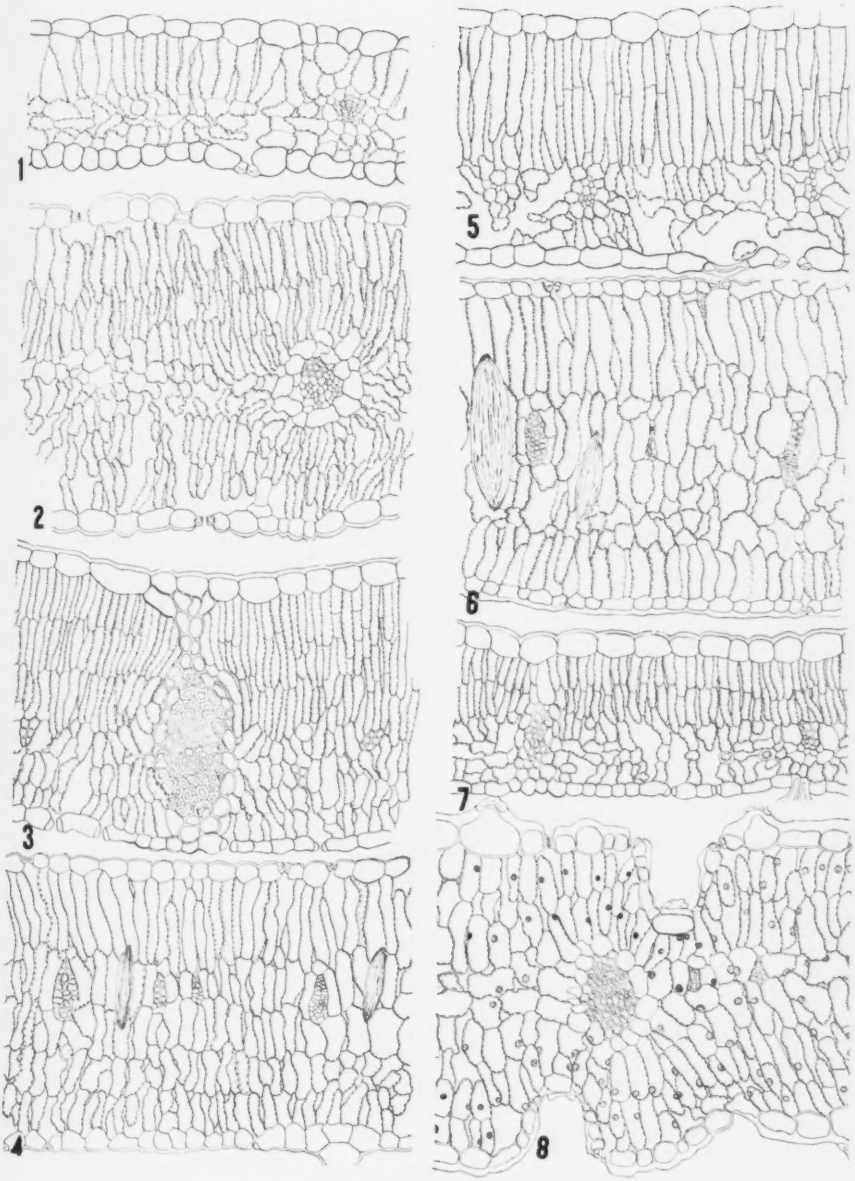
×80; fig. 13, *Geranium Richardsonii* (sun), ×80; fig. 14, *G. Richardsonii* (shade), ×80; fig. 15, *Senecio oblanceolatus*, ×48; fig. 16, *Gaura parviflora*, ×80.

PLATE XXII

FIGS. 17–24.—Fig. 17, *Andropogon furcatus*, ×80; fig. 18, *Thermopsis arenosa* (sun), ×80; fig. 19, *T. arenosa* (shade), ×80; fig. 20, *Populus tremuloides*, ×80; fig. 21, *Salix reticulata*, ×75; fig. 22, *Pentstemon glaucus*, ×80; fig. 23, *Cercocarpus parvifolius*, ×80; fig. 24, *Populus angustifolia*, ×80.

PLATE XXIII

FIGS. 25–33.—Fig. 25, *Euphorbia cuphosperma*, ×80; fig. 26, *Bistorta bistortoides*, ×80; fig. 27, *Streptopus amplexicaulis*, ×80; fig. 28, *Mertensia ciliata*, ×80; fig. 29, *Poa pratensis*, ×80; fig. 30, *Pentstemon unilateralis*, ×60; fig. 31, *Lactuca scariola*, ×80; fig. 32, *Chamaenerion angustifolium*, ×80; fig. 33, *Phleum pratense*, ×75.



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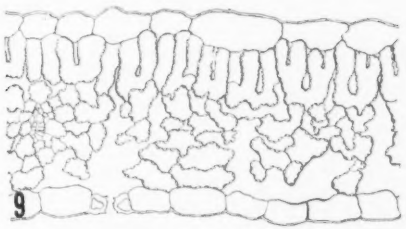

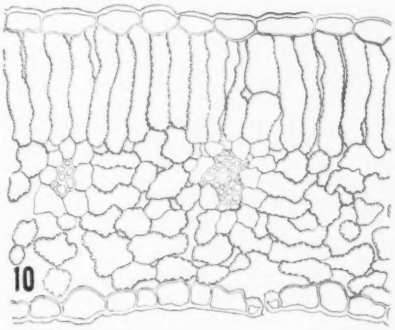

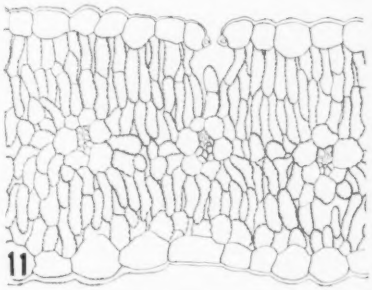

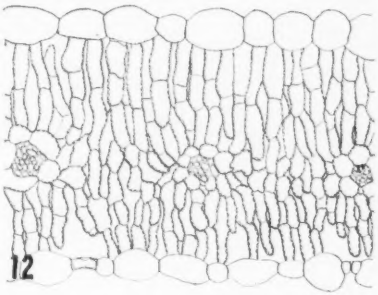

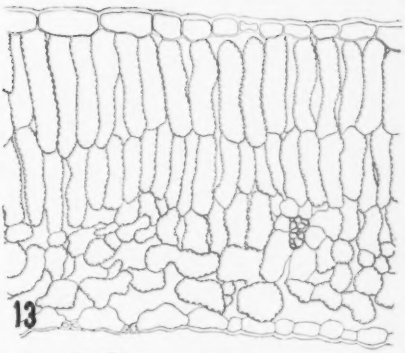

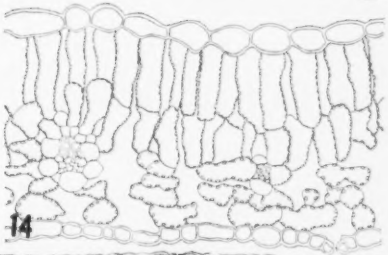

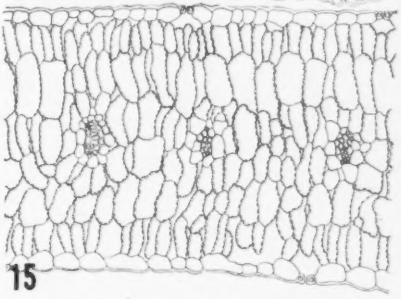

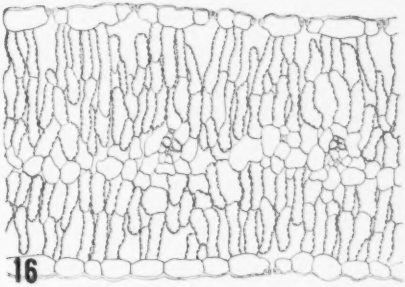

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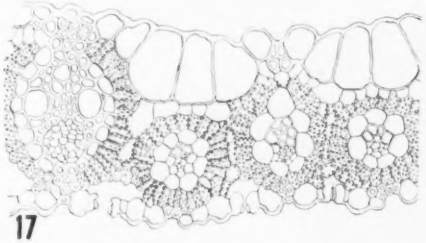

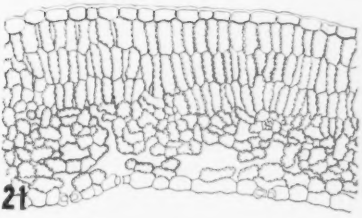

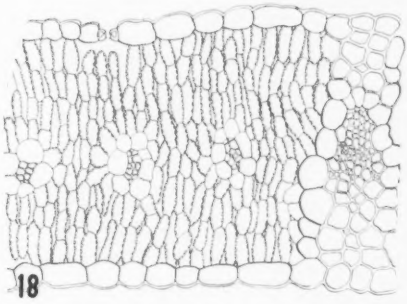

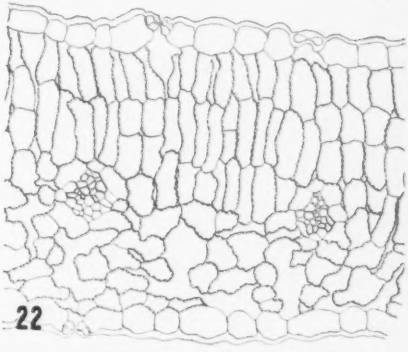

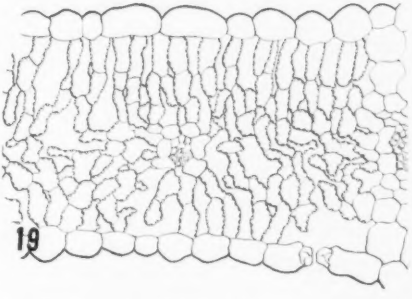

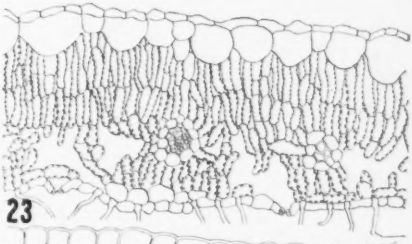

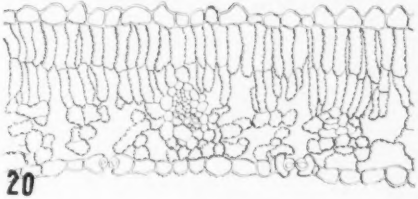

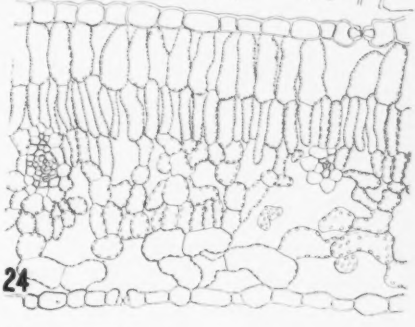

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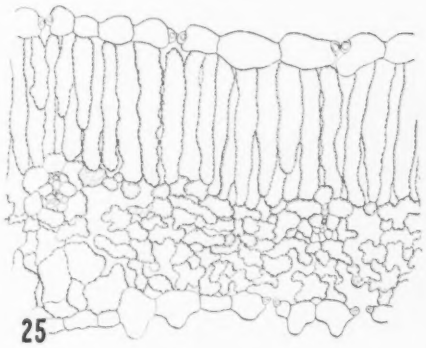

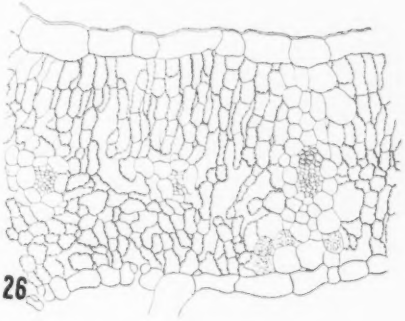

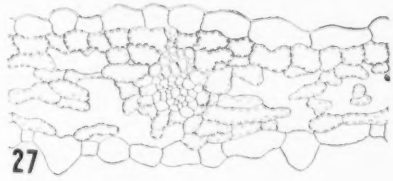

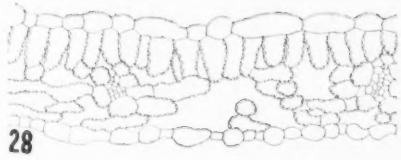

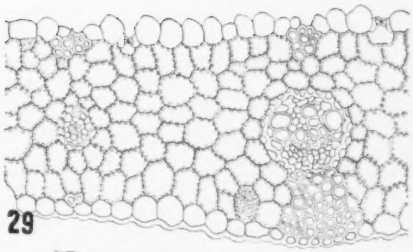

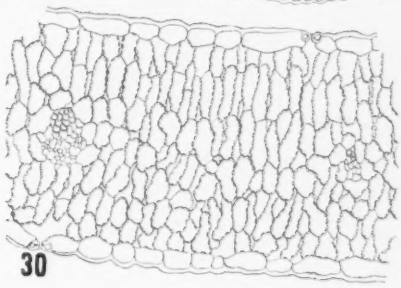

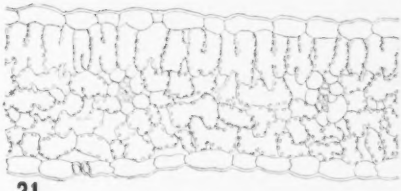

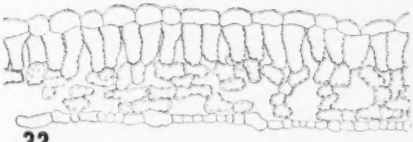

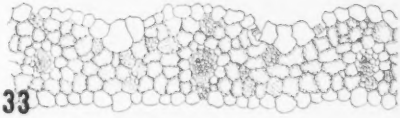

POOL on TRANSPIRATION

THE BARS OR RIMS OF SANIO[1]

JOSEPH D. HALE

(WITH PLATES XXIV–XXVI AND FIVE FIGURES)

Sanio's bar has lately been the subject of much discussion among botanists. It has been cited as incontrovertible evidence in support of diametrically opposed theories. By some it has been considered a character of utmost importance, and by others as something of only casual interest. In short, discussion over the bar has hitherto been largely unproductive.

The bar of Sanio derives its name from Dr. KARL SANIO (**6**), who described it from the stem of *Pinus sylvestris* in 1873. It consists of a rod-shaped thickening of the middle lamella between the radial walls of secondary wood tracheids, and is shown best by Haidenhain's stain. Miss GERRY (**3**) finds

> The bars . . . . take a blue stain, and stand out clearly from the red background obtained by general staining of tracheid walls with safranin. Often when they are absent or difficult to discern in the stem, they are found without difficulty in the root.

Text figs. 1 and 2 show the bars as they appear in the radial walls of tracheids from *Pinus* and *Ginkgo*. The middle lamella as it appears under the tracheid wall is indicated by stippling except at the thickenings or bars. The bars are represented in solid black. Miss GERRY's paper states that these thickenings of the middle lamella are found in the wood of all the conifers except the araucarians; they are present also in *Ginkgo* and in the Gnetales. This distribution is well known and admitted universally. In the illustrations of the bars, it will be noticed that the thickenings extend horizontally across the radial tracheid wall between pits or series of pits, and also that the bars are typically found in association with opposite pitting.

SANIO showed that early in the development of tracheids there appear in the radial walls thin spots which increase in size

[1] Contribution from the Laboratories of Plant Morphology of Harvard University.

with the tracheid itself. The tracheid walls between two of these thin spots on adjacent tracheids are fused and absorbed, and finally stretched into a thin membrane. On these attenuated areas

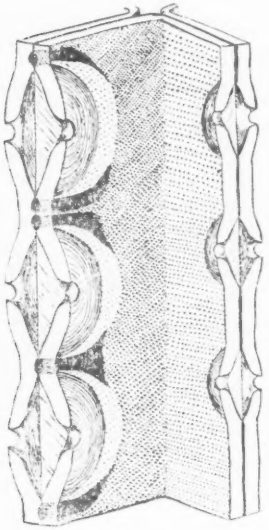

FIG. 1

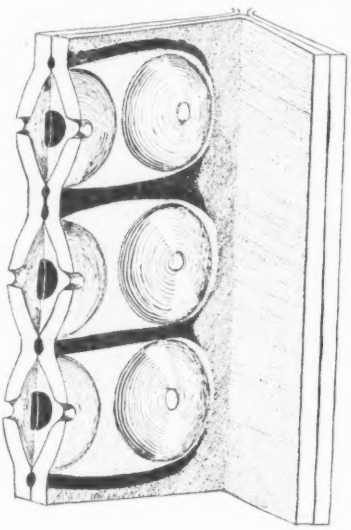

FIG. 2

FIGS. 1, 2.—Fig. 1, *Pinus:* bars or rims of Sanio comparable with those of *Ginkgo;* fig. 2, *Ginkgo:* bars or rims or Sanio in radial walls of tracheids between pits; bars absent in tangential walls.

pits are formed later, and SANIO designates these thin spots "Primordialtüpfeln" or "primary pit areas." BAILEY (1) states:

> It is evident from SANIO's figures and descriptions that he considered the primary pit areas, *Primordialtüpfeln*, to be separated by others in which the *Zwischensubstanz* is not entirely absorbed, and in which the outer cellulose layers are less attenuated. It should be emphasized, in this connection, that SANIO used the word *Querleisten*, cross-pieces or cleats, in referring to transverse thicker strips of the middle lamella between closely approximated primary pit areas, and the word *Umrissen*, contours, in referring to the upper and lower outlines of these areas. . . . . STRASBURGER showed very clearly that in *Pinus* and *Larix* SANIO's *doppelten Umrissen* may be the outlines of embossed portions of the middle lamella. In other words, he made it evident that when the primary pit areas are close together they are separated by a single transverse

thickening, but that when they are not closely approximated there may be two curved thickened strips, separated by a less heavily embossed area, between them.

In fig. 1 the primary pit areas are fairly close together, and the single transverse thickening between pits is seen (dark bands which frequently fork at each end). Fig. 4 shows a condition where the primary pit areas are "not closely approximated," and are separated by two thickened strips of middle lamella.

GROOM and RUSHTON (4) propose an amendment to abolish the term "bar," and suggest that the thickenings of the middle lamella be called "rims of Sanio." They state:

> The name "Sanio's rims" may be given to the structures causing the bandlike appearance. . . . . When the primary pit areas are in contact, the two contiguous Sanio's rims are naturally "fused" and form a band that is transverse and single, except possibly at the two lateral edges where the natural curvature of each original boundary of the area causes the band to fork. . . . . When young the actual marginal portion of the primary pit area does not thicken by deposits of lignified wall as soon as it does elsewhere (except on the pit-closing membrane), but thickens by successive deposits of pectic substance until a stage is reached when lignified wall-substance is deposited even over the now thickened rims of the primary pit area. Sanio's rims represent a system of rodlike or bandlike pectic thickenings of the middle lamella running transversely in the radial walls and linked here and there by slightly curved longitudinal bandlike similar thickenings (representing the lateral margins of the primary pit areas). See figs. 1, 3, 4, and 7. . . . . In tangential section, the appearance of these bands is seen to be due to a thickening of the middle lamella; where two successive superposed pits are close together, the thickening between them is single, but where the pits are some distance apart, there are two such thickenings, one near each pit.

The foregoing citations comprise most of our knowledge of the development of the so-called "bars" or "rims" of Sanio. Their occurrence on the radial walls of secondary wood tracheids as transverse thickenings of the middle lamella has already been emphasized. Their distribution has been mentioned as confined to the Ginkgoales, the Coniferales (except the araucarians), and the Gnetales. They are present, however, in the cone axis of the araucarian conifers, and this fact has led to conflicting generalizations.

There is the clearest anatomical evidence that the conifers are co-descendants with the Ginkgoales from the Cordaitales. The

Cordaitales had alternate pitting throughout when bordered pits were present, and show no evidence whatever of bars of Sanio. *Ginkgo* has opposite pitting in its stem and root wood, with well developed bars of Sanio, but in certain primitive regions it retains some of the characters of its ancestors, the Cordaitales. The first formed secondary wood tracheids of the stem of *Ginkgo* have alternating pits and no bars of Sanio. In fact, only when the third or fourth tracheid of the secondary wood is reached do we find normal opposite pitting and bars of Sanio (text fig. 3, and fig. 2). The

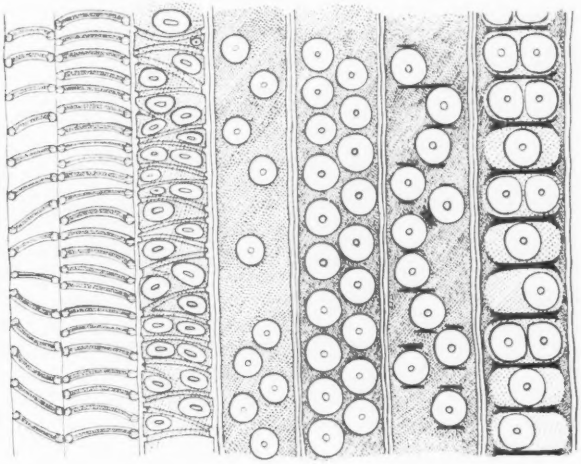

FIG. 3.—*Ginkgo:* radial section, showing transition in tracheary sculpture from primary wood to secondary wood of first annual ring of stem; first tracheids of secondary wood show Cordaitean character of alternate pitting and absence of bars of Sanio.

presence of Cordaitean characters in primitive regions of *Ginkgo*, when taken in consideration with other anatomical features, establishes the sequence of *Ginkgo* between the Cordaitales and the Coniferales.

The araucarian stem and root wood has alternate pitting and no bars of Sanio, a condition exactly like that in the Cordaitales, but we hesitate to place it near these ancient forms, because in the araucarian cone, axis, etc., vestigial bars of Sanio are found, accompanied by opposite pitting, a phenomenon which suggests that the ancestors of the araucarians had bars of Sanio in their stem wood.

In consideration of these facts, the other conifers, particularly the Abietineae, are logical ancestors of this subtribe, and since fossil araucarians are found with such abietinean characters as resin canals like those of *Pinus*, the Abietineae have been considered as probable ancestors of the araucarians.

The bar of Sanio in the araucarian cone axis is peculiar in that it usually extends only the width of a single pit (figs. 6, 8), and frequently does not cross a whole series of pits as in the conifers and in *Ginkgo* (figs. 1, 3, 4). The Gnetales are usually regarded as having come from the same primitive stock as the Angiosperms and show many conifer characteristics, such as opposite pitting and bars of Sanio (fig. 7).

There has been some discussion as to the distribution of these bars. Their occurrence had never been described anywhere save in the radial walls of secondary wood elements of *Ginkgo*, the conifers, and the Gnetales, until in 1915 SIFTON (7) reported the discovery of bars of Sanio in primary wood of the petiole of *Cycas revoluta*. He regarded the presence of these supposed bars in *Cycas* as invalidating the theory of abietinean ancestry for the araucarians. He says:

> The discovery of bars or rims of Sanio in the cycads must either nullify their value as evidence of the derivation of the araucarians from the Abietineae, or indicate that the Abietineae are also ancestral to the cycads, a position which can hardly be assumed. Moreover, the study of the rims in different regions of the Abietineae indicates that the ancestral type of bar as found in the cycads and araucarians has become specialized in the higher conifers, the original type persisting only in the primitive regions, the cone axis and the root. Thus the discovery of the bar in cycads has, besides confirming the primitive character of this structure in araucarians, made apparent its significance in the different regions of the Abietineae.

SIFTON regards the short bars of araucarian cones as a primitive type. The long bars of the other conifers, which reach entirely across the radial facets of tracheids, he believes are more advanced in development from an ancestral short type of bar. *Ginkgo*, however, has longer bars than many conifers, especially in its root. As *Ginkgo* is considered a much more primitive form than the araucarians, the theory which regards short araucarian bars as primitive is not convincing.

SIFTON shows illustrations of only one part of a single section of *Cycas revoluta* to support his theory. The tracheid of *Cycas* which illustrates the bars is one from which part of the wall was cut away. The remaining wall shows only a single vertical row of pits. The slanting cut of the knife has left an edge of lignified cell wall projecting somewhat beyond the overlying middle lamella. The middle lamella consequently shows as a dark band running out between pits and terminating at the line of the cut with sometimes forking endings. This middle lamella band, however, projects not only above and below the pit, but when not cut away it follows around the margin of the pit, conforming absolutely to it. The true bar of Sanio characteristically does not conform to the pit margin, but runs directly across the tracheid wall. Even in araucarian cones (figs. 6, 8) the bar is confined to the upper and lower margin of the pit, and has abrupt lateral endings which usually fork. The alleged bars of *Cycas* described by SIFTON conform absolutely to the outline of the pit border. The pit membranes are thin and take a much lighter stain than the middle lamella which surrounds them. Consequently, a photograph shows the pits as light circles surrounded by the dark anastomosing middle lamella, which conforms to the shape of the pit border (figs. 11, 12, 13). This anastomosing middle lamella is typical not only of the cycads, but in fact of all tracheids, no matter what the type of pitting may be (figs. 9, 10, 14, 16, 17), from the lowest vascular plants to the highest. On the other hand, the bar of Sanio is a special thickening in the middle lamella of limited occurrence, as has been shown, being found only in secondary wood of certain species. It follows, of course, that it is not a feature of primary wood at all.

The upper right hand corner of fig. 13, the tracheid of *Encephalartos*, shows much the same condition as the *Cycas* tracheid figured by SIFTON. A part of the middle lamella has been cut away and the lignified wall beneath remains. The cut ends of the middle lamella show forking endings, of course, but the lower part of the tracheid correctly represents the middle lamella conforming to the pit outlines. If the mere anastomosis of the middle lamella between scalariform and similar pits is regarded as the bar of Sanio, the

distribution of the bar is universal with the distribution of pitting and the occurrence of the middle lamella. Indeed, the bar would of necessity be present from the lowest to the highest vascular plants wherever there were tracheids. On the other hand, if the bar is something more than the mere undifferentiated middle lamella, if it is a special thickening of the middle lamella, its distribution is certainly limited to the middle lamella of the secondary wood of certain particular groups.[2]

BAILEY believes the bar of Sanio to be merely a "concomitant of reduction in tracheary pitting," and asserts:

> Bars or rims of Sanio, which occur in certain Pteridophyta and Angiospermae, as well as in many Gymnospermae, are concomitants of processes of modification or reduction in tracheary pitting.

Figs. 9 and 10 show vessels of *Pteris aquilina*, a form chosen by BAILEY to illustrate the bars of Sanio in Pteridophyta. Between the long narrow pits extends, naturally, the middle lamella, which shows in the photographs as a dark band in sharp contrast with the lighter shade of the pit border. It is this part of the middle lamella between the pits which BAILEY describes as "the peculiar bandlike thickenings of the middle lamella (so-called bars or rims of Sanio) which occur in certain Pteridophyta."

There are similar cases of scalariform pitting in all the Filicales. Of the numerous examples, some of the most common species were selected, namely, *Pteris aquilina*, the common bracken fern, and *Dicksonia punctilobula*. In these quite typical forms, pitting of the usual scalariform type is abundant. Between the long scalariform pits the middle lamella shows as a sharp band in contrast with the lignified wall, exactly as in *Pteris* (fig. 10). Those regions of the middle lamella between the scalariform pits, however, do not resemble the rims of Sanio, for they are not thicker than the

[2] The bars of Sanio in araucarian cone axes, on which JEFFREY partly based his theory of Abietinean ancestry of the conifers, occur in the secondary wood tracheids of the transitional region of the araucarians, and not in the primary wood, as SIFTON (8) seems to indicate. JEFFREY (5) states "Bars of Sanio are clearly present in the secondary wood tracheids adjacent to the primary wood of the cone axis in these two genera" (*Agathis* and *Araucaria*). SIFTON's recent discovery of a bar in the secondary wood of *Dioon spinulosum* is not convincingly exemplified, and is subject to the same criticism as is his bar of Sanio in primary wood.

middle lamella elsewhere. There are no rodlike thickenings between the scalariform pits of fern tracheids at all comparable with the rims in the Coniferales. In every case where there is scalariform pitting, the middle lamella extends in a uniform layer on all sides of the pit membranes, and is nowhere interrupted by rodlike thickenings (text fig. 4 and figs. 9, 10, 14, 17). The middle lamella, anastomosing and conforming to the outlines of the pits, has again been mistaken for the bar of Sanio.

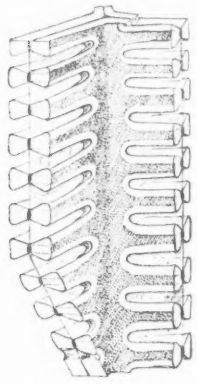

FIG. 4.—Fern: scalariform type of pitting; complete absence of thickenings in middle lamella.

Besides stating that bars of Sanio are widely distributed, BAILEY says:

> The comparative anatomy and ecology of the Pteridophyta, Gymnospermae, and Angiospermae afford considerable evidence which suggests that the types of unconformity and peculiar bandlike thickenings of the middle lamella (so-called bars or rims of Sanio) which occur in certain Pteridophyta and Angiospermae, as well as in many Gymnospermae, are concomitants of processes of modification or reduction in tracheary pitting.

The evidence for this view has previously been discussed somewhat, but the facts against such a view seem more important and numerous than those which can be summoned in its favor. The statement that bars of Sanio are the remains of a scalariform structure of the middle lamella which persists from an ancestral scalariform pitted tracheid is not convincing. First, the phylogenetic evidence is clearly against such a view, since conifers, Ginkgoales, and Gnetales are groups which actually (Gnetales) or for long periods in the past have been characterized by alternating pitting and complete absence of scalariform structure in the secondary wood (Ginkgoales and Coniferales). Furthermore, the developmental evidence does not favor the conclusion drawn by BAILEY, since, as has been shown in the case of *Ginkgo*, a very important transitional form, the bars of Sanio only make their appearance with the establishment of opposite pitting, a condition clearly preceded in the secondary tracheids near the primary wood by tracheids with

alternating pitting and absence of anything resembling bars of Sanio. As has been shown, the structural evidence is absolutely against the confusion of the horizontal portions of middle lamella occurring in the case of scalariform pitting (from the lowest forms to the highest) with structures long recognized as bars of Sanio. From the point of view of phylogeny, or the historical evidence, we must consider the species in which the bars are best developed, and also look into the structure of the antecedents of these species.

Ginkgo, the form universally accepted as more primitive than the conifers, has well developed bars of Sanio. It has, however, no scalariform pitting in the secondary wood, so that there is no evidence that the bar could have arisen from a scalariform structure in the middle lamella in *Ginkgo* itself. The forms universally conceded to be ancestral to *Ginkgo*, the Cordaitales, had scalariform pitting in the transitional region, but this scalariform sculpture passed into alternate pitting instead of opposite, and no bars of Sanio have been described in any of the Cordaitales, although well preserved material is abundant. It may further be noted that in many Cordaitales the pitting is very lax, so that there is no difficulty arising out of crowded pores preventing the presence of bars of Sanio. In spite of this situation, bars do not make their appearance in loosely pitted Cordaitean woods. As JEFFREY has shown, bars of Sanio are characteristically correlated with opposite pitting. The bar in *Ginkgo* cannot be considered a left-over structure from Cordaitean scalariform pitting, because the Cordaitales had developed alternating pitting toward the very beginning of their existence and before *Ginkgo* was evolved. Moreover, the primitive regions of *Ginkgo* have Cordaitean rather than scalariform structure. Alternating pitting is present in conservative regions, which suggests ancestors with alternating pittings. Text fig. 3 shows the structure of the primary wood and first formed secondary wood of *Ginkgo*. There is a sharp transition from ringed tracheids of the primary wood to the pitted tracheids of secondary wood, quite unlike the gradual transition in the cycads (text fig. 5). There is, following the ringed tracheid, a pitted tracheid which has ringlike thickenings, and this in turn is followed by pitted elements. It should be noticed especially that the first two tracheids

following the ringed and pitted tracheids have alternating pits and no bars of Sanio, a clear Cordaitean feature. The conifers have transitions in their first annual rings which show complete absence of scalariform pitting, and it is obvious that this is another important break in the chain of evidence if we explain bars of Sanio as a relic of scalariform pitting. In the woods where bars of Sanio are best developed there is no scalariform pitting, and further, there is no scalariform pitting in the secondary wood of

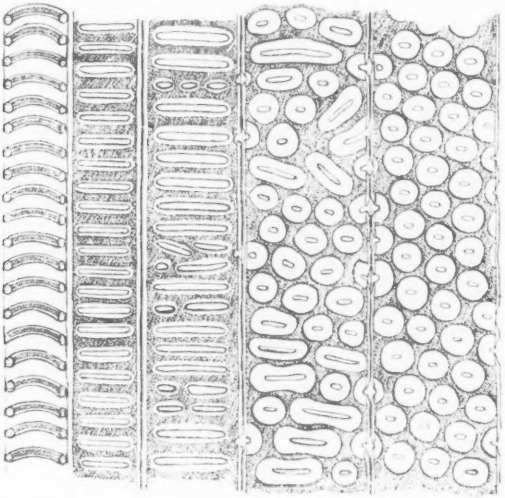

FIG. 5.—Cycad: radial section, showing transition in tracheary pitting from scalariform to bordered, with no thickenings in middle lamella resulting.

their ancestors from which the bars of Sanio might have been derived in accordance with the hypothesis of BAILEY.

From the point of view of development, the ferns and cycads are the logical types to study, since they show striking examples of clear transition from scalariform to round-bordered pits. The petiole of *Cycas*, *Dioon*, *Encephalartos*, etc., illustrate in radial sections a transition from ringed and spiral protoxylem to scalariform and bordered-pitted metaxylem, but with never a sign of bars of Sanio. The middle lamella conforms to the pit outline everywhere, and in no case are there any thickened horizontal bands

comparable with the bars of Sanio. Fig. 11 represents a tracheid of *Cycas revoluta* which shows a transition from scalariform to round-bordered pits in the same element. The middle lamella appears as dark bands behind the lighter foreground of lignified tracheid wall, the middle lamella always bordering the pit membranes. In the center of the tracheid the middle lamella extends across a former scalariform pit area in two wide bands, connecting the upper and lower margins and separating the pit area into three round-bordered pits. Structures of this kind are never observed in the case of true bars of Sanio. This in itself is a cogent argument against the origin of bars as appearing in transition from scalariform to pitted sculpture of the tracheid walls, for always in such transitions there are many anastomosing strands of middle lamella running in every direction and conforming to the pit margins. The true bars of Sanio occur horizontally and are not united by vertical cross members. Moreover, the outline of a scalariform structure is very rarely retained in the transition process, and the pits which occupy an area which was once a scalariform pit are arranged anew in some alternating alignment. Figs. 11–14, 17 represent various steps in the transition of scalariform pits to round-bordered pits. The middle lamella conforms to the pit outlines in every case, and the ultimately alternating arrangement is also a notable feature in these illustrations. Fig. 15 is a metaxylem tracheid of *Dicksonia punctilobula*, and will serve to represent the transition process in ferns. The tracheid represents an advanced stage of opposite bordered pitting, and here, if anywhere, when the pits are still opposite and well spaced, we should find bars of Sanio if they exist in primary wood as results of transition in tracheary pitting. The fact that bars are conspicuously absent completes the developmental evidence against the theory of persistent scalariform structures.

Bars of Sanio have never been described in any specimen of primary wood of conifers. If bars of Sanio were widespread in the Pteridophyta, which have for the most part only primary wood, and in the primary wood of Archi-Gymnospermae as SIFTON asserts, there would be every reason to expect bars of Sanio in the primary wood of the conifers, since they are so well developed there in the

secondary wood. Their absence in the primary region of conifers apparently is valid evidence against the theory of bars from the bands of middle lamella necessarily occurring in the case of scalariform pitting. There seems to be absolutely no valid evidence as to any relationship between the bars of Sanio and scalariform pitting. They are in fact uncorrelated phenomena. JEFFREY maintains that the bar of Sanio is a valuable character of phylogenetic significance in determining the affinities of the araucarian conifers. If the bars are of use in diagnosing coniferous affinities, it is reasonable to expect that the occurrence of these bandlike thickenings of the middle lamella should persist in some of the recognized primitive regions of the higher angiosperms. The occurrence of Sanio's rims in the Gnetales, which are probably closely related to the Angiosperms, is therefore to be expected.

The Betulaceae have long been regarded as a primitive group of Angiosperms, and, in the hope of finding some relic of the bars or rims of Sanio, specimens of *Corylus*, *Ostrya*, *Carpinus*, *Betula*, and *Alnus* were examined. All these genera show some evidence of bandlike thickenings of the middle lamella in radial walls of the secondary wood. The bars or rims of Sanio are much more prominent in the roots of the Betulaceae than in the stems. Especially in large tracheids of the spring wood rather than in the vessels, the bars show to best advantage. The root wood of plants is generally formed of larger and thinner walled tracheary elements than is the stem wood, and this fact probably facilitates demonstration of the bars. The bars, however, are not confined to the Betulaceae. The tracheids of root wood of the Magnoliaceae are characterized frequently by the presence of Sanio's rims. Tracheids from the root of *Cercidiphyllum japonicum* show as distinct bars of Sanio as does *Betula papyrifera*. *Sassafras* and *Catalpa* show bars of Sanio especially in their roots, as do *Fagus* and *Fraxinus*.

Of the higher forms which exhibit bars of Sanio, *Fraxinus* is the most advanced which has been studied in this connection. In *Fraxinus* the bars lose the sharp and distinct structure which they exhibit in the conifers, and spread out over a somewhat larger area (figs. 20, 25). Apparently the rims of Sanio die out in the higher Angiosperms, and the indistinct structure of the rims

in *Fraxinus* and other genera is evidence of the vestigial character of the bars in these forms. The bars persist in *Fraxinus* only in the radial walls of the large tracheid-like fibers of spring wood at the beginning of the annual ring. In the conifers the bars are most marked in the spring tracheids, and in the higher Angiosperms large elements of the spring wood become the regions of the final persistence of these probably vestigial structures.

In the Betulaceae, however, the bars are most prominent. Of this family *Alnus* has the most distinct bars of Sanio. *Alnus japonica*, *A. maritima*, and *A. rhombifolia* show the bars to best advantage. It is very striking that the bars of Sanio are barely noticeable in the stems of these species, even with the most minute search. In the roots, bars are immediately prominent (figs. 18, 21, 24). The root of *A. maritima* has probably the best developed bars of the three, but it is closely followed in this respect by *A. japonica*. *A. rhombifolia* has fewer bars than either of the two foregoing species. It is an interesting fact that *A. japonica* and *A. maritima*, although widely separated in distribution, have been considered closely related. BRITTON (2) says of *A. maritima*, that it is "closely related to *Alnus japonica* of Northeastern Asia and perhaps not specifically distinct from it." *A. maritima*, *A. japonica*, and *A. rhombifolia* are all tree alders and the only species of those examined which showed striking occurrence of bars of Sanio. The others which did not show the bars were *A. incana*, *A. rugosa*, *A. mollis*, and *A. sinuata*, all shrubby species.

Bars of Sanio in the Betulaceae seem to be vestigial characters, persisting chiefly in the primitive regions. If the arboreal type is a primitive one in the case of the alders, then the occurrence of the bars in the root wood of tree alders is all the more striking. The vestigial structure of the bars in higher Angiosperms, when considered in connection with their occurrence in primitive regions of lower Angiosperms, would seem to indicate that the bars of Sanio in these forms are an inheritance from the Coniferales and the Gnetales.

Conclusions

The evidence submitted, supported by the accompanying illustrations, seems to warrant rather definite conclusions. The

theory put forward by SIFTON and BAILEY as to the relationship between the bands of middle lamella prominent in the case of scalariform pitting and the bars of Sanio found in the secondary wood of Ginkgoales, Coniferales, Gnetales, and the Angiosperms, seems to be without sufficient foundation. There are no transitions between such structures and true bars of Sanio. Furthermore, bars of Sanio are not found in forms which possess ancestrally scalariform pitting in their secondary wood, namely, the cycads and other primitive Gymnosperms. On the other hand, bars of Sanio are well marked in groups such as the Gnetales and Coniferales, which possess ancestrally alternating pitting in their secondary wood. It is pertinent in this connection to point out that the structures designated by SIFTON and BAILEY as the primitive condition of the bar of Sanio occur wherever scalariform pitting makes its appearance. For example, scalariform vessels of the Vitaceae are universally characterized by the presence of horizontal bands of middle lamella which lie between the broad scalariform pits. In an even higher group, the Compositae, a similar condition is of frequent occurrence, as shown in fig. 16, illustrating the structure of the wood in *Helianthus annuus*. It thus appears that the structures interpreted by SIFTON and BAILEY as the primitive type of the bars of Sanio are an optical phenomenon which always and necessarily accompanies scalariform pitting whether in primary or secondary wood, in lower or higher forms (fig. 22). If the structures brought into prominence by SIFTON and BAILEY, but which are by no means new to science, are to be regarded as true bars of Sanio, then these structures are of no diagnostic importance. On the other hand, the structures confined to the radial walls of water-conducting elements of secondary wood and consisting of definite transverse thickenings of the middle lamella which occur in the Ginkgoales, Coniferales, Gnetales, and Angiosperms, have nothing whatever to do with the scalariform thickenings, since they find their expression in forms which either actually (Gnetales) or in the past (Coniferales and Ginkgoales) are characterized by the possession of alternate pitting. It follows that scalariform pitting has no relation whatever to the development of the structures which are properly regarded as bars of Sanio.

Summary

1. The structures described by SIFTON and BAILEY in primary wood of ferns and Gymnosperms do not properly come under the category of bars of Sanio

2. True bars of Sanio, consisting of horizontal thickenings of middle lamella, are found only in secondary wood, and are absent in primary elements.

3. True bars or rims of Sanio characteristically occur in the secondary wood only of the Ginkgoales, Coniferales, Gnetales, and Angiosperms.

4. They are of diagnostic importance for these groups.

This investigation was carried on in the Harvard Laboratories of Plant Morphology under the direction of Professor E. C. JEFFREY.

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MONTREAL, CANADA

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EXPLANATION OF PLATES XXIV–XXVI

PLATE XXIV

FIG. 1.—*Ginkgo biloba:* radial secondary xylem of stem, showing bars or rims of Sanio and opposite pitting.

FIG. 2.—*Ginkgo biloba:* radial section, showing transition from primary to secondary xylem in first annual ring of stem.

FIG. 3.—*Ginkgo biloba:* radial facet of tracheid, showing bars or rims of Sanio and opposite pitting.

FIG. 4.—*Taxodium distichum:* radial secondary xylem of stem, showing bars or rims of Sanio.

FIG. 5.—*Pinus rigida:* tangential secondary xylem of root, showing bars or rims of Sanio in section.

FIG. 6.—*Araucaria Bidwillii:* radial section of cone axis, showing bars or rims of Sanio.

FIG. 7.—*Gnetum scandens:* radial facet of tracheid, showing bars or rims of Sanio.

FIG. 8.—*Araucaria imbricata:* radial section of cone axis, showing bars and rims of Sanio.

PLATE XXV

FIGS. 9, 10.—*Pteris aquilina:* facet of tracheid.

FIG. 11.—*Cycas revoluta:* tracheid facet, showing transition from scalariform type of pitting.

FIG. 12.—*Macrozamia spiralis:* tracheid facet, showing transition from scalariform type of pitting.

FIG. 13.—*Encephalartos horridus:* tracheid facet, showing transition from scalariform type of pitting; note anastomosing middle lamella.

FIGS. 14, 15.—*Dicksonia punctilobula:* tracheid facet, showing transition from scalariform type of pitting.

FIG. 16.—*Helianthus annuus:* vessels showing anastomosing middle lamella.

FIG. 17.—*Dicksonia punctilobula:* tracheid facet of transition region of opposite bordered pits.

PLATE XXVI

FIG. 18.—*Alnus japonica:* radial secondary xylem of root, showing bars or rims of Sanio.

FIG. 19.—*Alnus maritima:* tangential section of root; secondary xylem showing bars or rims of Sanio.

FIG. 20.—*Fraxinus oregona:* radial secondary xylem of stem, showing bars or rims of Sanio.

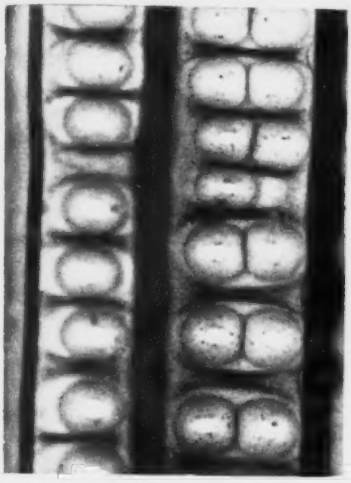
FIG. 21.—*Alnus rhombifolia:* radial secondary xylem of root, showing bars or rims of Sanio.

FIG. 22.—*Magnolia Fraseri:* facet of root vessel, showing anastomosing middle lamella.

FIG. 23.—*Fraxinus nigra:* radial secondary xylem of stem, showing bars or rims of Sanio.

FIG. 24.—*Alnus maritima:* radial facet of secondary xylem tracheid, showing bars or rims of Sanio.

FIG. 25.—*Fraxinus oregona:* tangential section, secondary xylem of stem, showing bars or rims of Sanio.



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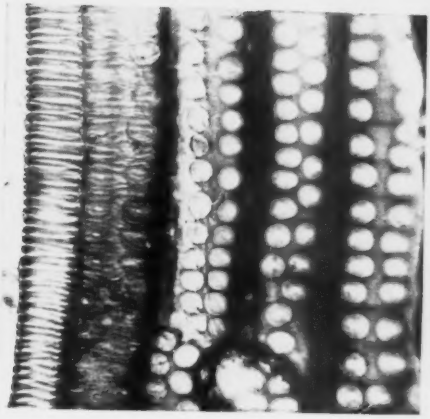

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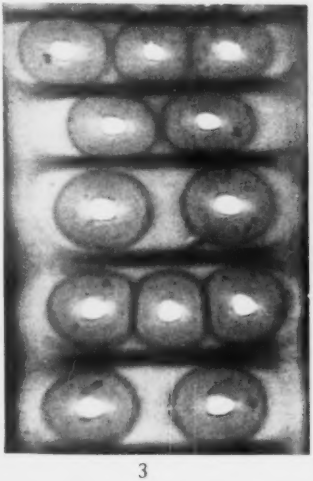

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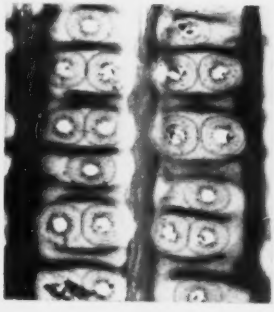

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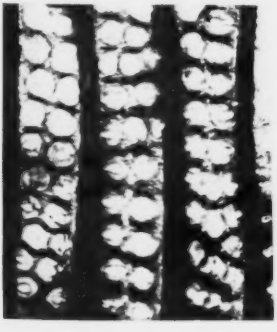

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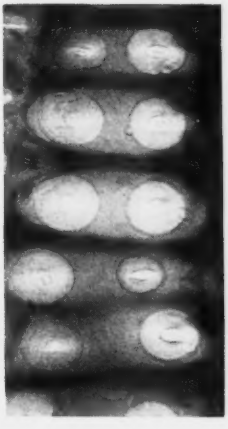

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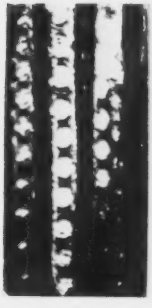

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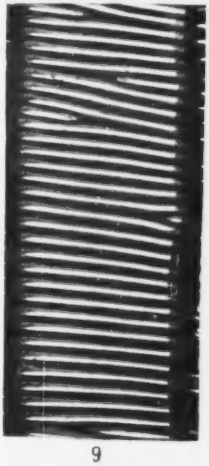
HALE on BARS OF SANIO



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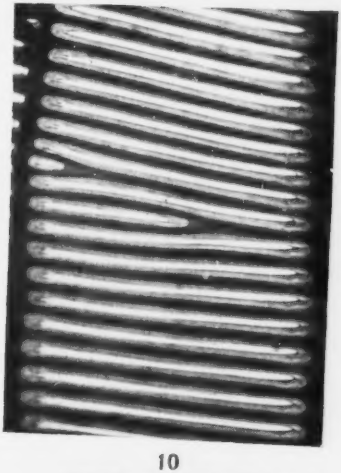

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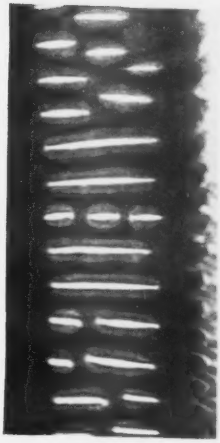

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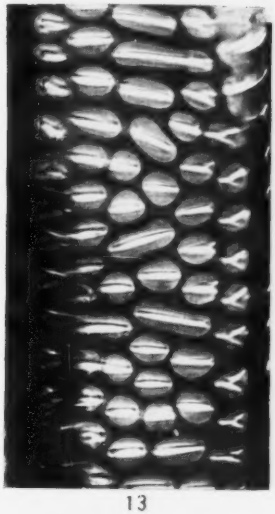

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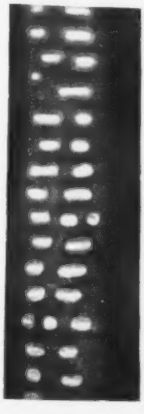

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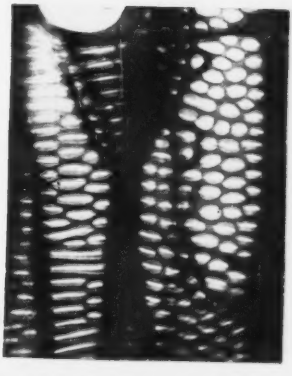

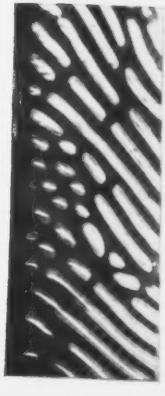

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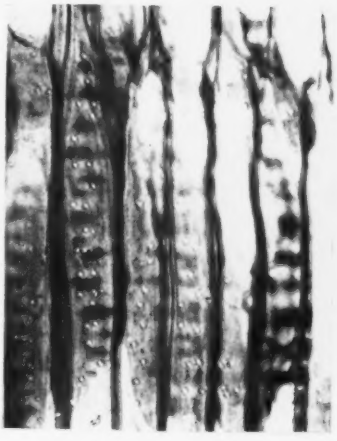

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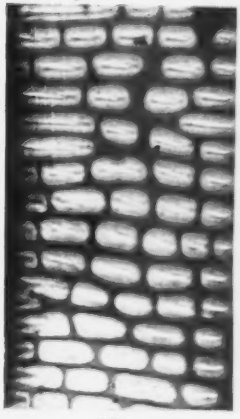

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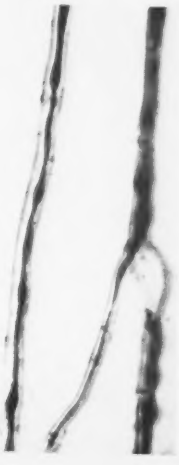

HALE on BARS OF SANIO

INFLUENCE OF GLUCOSE AND FRUCTOSE ON GROWTH OF FUNGI[1]

J. M. BRANNON

As reported in a previous paper (**2**), the view has been maintained that glucose is primarily a respiratory sugar, and fructose a tissue former. Among those who have expressed this view are LINDET (**19**) and MOLLIARD (**22**). LINDET states that yeast utilizes fructose for tissue formation to a greater extent than it does glucose. MOLLIARD grew *Sterigmatocystis niger* on a slightly acid medium containing sucrose. When not enough nitrogen was present to utilize all the sugar growth was retarded, but respiration continued. In time all the glucose was exhausted, but much of the fructose was still in the solution. The medium used was a modified Raulin's solution. With a neutral medium, MOLLIARD thinks that more mycelia would have been produced and both sugars utilized equally. From this he concludes that levulose is the predominant sugar in tissue formation. CZAPEK (**7**) found that of the six sugars he used with *Aspergillus niger*, fructose produced the greatest growth, and the remaining five sugars were used in the following order: l-xylose, d-galactose, d-glucose, l-arabinose, and d-mannose. HAWKINS (**14**) grew *Glomerella coagulata* on media containing either glucose, arabinose, or xylose. On the media containing glucose he got an average dry weight of 348.3 mg., on the media containing arabinose he got an average dry weight of 380.2 mg., while the media containing xylose gave a dry weight of 387.8 mg.

Methods

The fungi were grown on Czapek's solution, as used by DOX (**8**). Stock solutions of this medium were prepared and the portions to be used were measured out and the sugars added. The sugars used were of a high degree of purity. In most of the experiments globe flasks of 250 cc. capacity were used as culture vessels. These flasks are especially good because when about half filled a large

[1] Portion of a thesis presented to the Graduate School of Cornell University in partial fulfilment for the degree of Doctor of Philosophy.

TABLE A

INFLUENCE OF SUGAR ON GROWTH OF FUNGI

ORGANISM	DURATION IN DAYS	CONCENTRATION	NUMBER OF CULTURES OF EACH SUGAR	DRY WEIGHT OF MYCELIUM IN MG.			
				Fructose	Dextrose	Sucrose	Maltose
Aspergillus niger	61	2 per cent	8	259 ± 8	275 ± 9	322 ± 24	242 ± 11
Aspergillus niger	60	2 per cent	8	559 ± 5.2	534 ± 3	557 ± 20.6	549 ± 9.3
Aspergillus niger	.....	1 per cent	11	273 ± 3.3	247 ± 7.74	.....	.....
Aspergillus niger	45	7 per cent	9	1534 ± 36	1315 ± 28	.....	.....
Penicillium Camembertii	70	1 per cent	6	216 ± 6.8	163 ± 12	156 ± 10	.....
Penicillium Camembertii	.....	2 per cent	10	144 ± 4.8	109 ± 5	.....	.....
Penicillium Camembertii	.....	5 per cent	11	1039 ± 63	791 ± 31.2	.....	.....
Penicillium Camembertii	70	1 per cent	8	198 ± 9.4	183 ± 4	210 ± 10	201 ± 9.3
Fusarium sp.	42	1.5 gm. dextrose 1.7 gm. levulose	9	364	374 ± 8.9	.....	.....
Cylindrocladium scoparium	45	1.5 gm. dextrose 1.7 gm. levulose	7	466 ± 14.6	472 ± 20.9	.....	.....

surface of the culture medium is exposed. One hundred cc. of the culture solution was put into each flask and the flasks and culture solutions sterilized for forty minutes at ten pounds pressure. The flasks were inoculated by adding 5 cc. of a suspension of the spores, and then incubated at 20° C. This temperature was maintained in all cases excepting two, when a temperature of 30° was used.

A considerable number of experiments were conducted, and in some of these sugar determinations were made, but in these experiments such irregularity of growth was obtained that the results were of no value. Table A incorporates the results of ten different experiments on the influence of these two sugars on growth. Only the average results are reported, with the probable error calculated for each according to Bissel's formula.[2]

The experiments with fungi are summarized in table A. In comparing glucose and fructose, it will be noted that no significant differences are found in experiments 1, 8, 9, and 10. In experiments 2, 3, 4, 5, 6, and 7, the approximate odds in favor of the greater growth on fructose are 140–1, 22–1, 519–1, 95–1, 600–1, and 64–1, respectively. In no case is maltose superior to fructose, while in experiments 1 and 5 sucrose is significantly better than fructose.

Experimental data

EXPERIMENT 1.—A species of *Penicillium* was used in this experiment, which lasted for ten weeks. The filtrates gave no tests for sugar. The results are shown in table I.

TABLE I

YIELDS (IN MG.) OF *Penicillium* SP. ON CZAPEK'S MEDIUM, CONTAINING EITHER 1 PER CENT LEVULOSE, DEXTROSE, OR SUCROSE

Levulose	Dextrose	Sucrose
98	118	113
100	133	90
104	103	107
108	120	105
115	.....	103
.....	.....	101
Average: 105	118	104

[2] MELLOR, Mathematics for students of chemistry.

EXPERIMENT 2.—In this case *Penicillium Camembertii* was used. The growth rate of this fungus was slower than the species used in the first experiment. The results are shown in table II.

TABLE II

GROWTH (IN MG.) OF *Penicillium Camembertii* ON CZAPEK'S SOLUTION CONTAINING 1 PER CENT SOLUTION OF EITHER DEXTROSE, SUCROSE, OR LEVULOSE; PERIOD OF TEN WEEKS

Dextrose	Sucrose	Levulose
151	82	246
165	173	208
221	158	200
202	150	212
99	190	.....
143	177	.....
Average: 163	156	216

EXPERIMENT 3.—This experiment is similar to no. 2, except that there were a few cultures containing maltose. The results are given in table III.

TABLE III

YIELDS (IN MG.) OF *Penicillium Camembertii* GROWN ON CZAPEK'S SOLUTION CONTAINING EITHER 1 PER CENT DEXTROSE, LEVULOSE, SUCROSE, OR MALTOSE; PERIOD OF TEN WEEKS

Dextrose	Levulose	Sucrose	Maltose
193	248	268	119
153	226	234	191
179	148	278	233
182	220	205	262
172	229	159	226
214	197	232	216
178	176	156	206
193	144	170	176
.....	192	192	176
Average: 183	198	210	201

EXPERIMENT 4.—This experiment is similar to the preceding three, except that the solutions were titrated with N/10 alkali after the fungus had been filtered off. The results are given in table IV.

CURRIE (6) has shown that citric and oxalic acid are produced when *Aspergillus niger* is grown on a culture medium containing sucrose. BUTKEVITSCH (5) found that when *A. niger* was grown on a culture solution containing sugars the medium became acid, due to oxalic acid. JOST (15), quoting the work of WEHMER and EMMERLING, showed that the amount of oxalic acid produced seldom exceeds 0.3 per cent, but if this acid is neutralized the fungus would again produce acid. If *Aspergillus* was grown on a nutrient solution containing 1.5 gm. of either tartaric or citric acid, about 0.2 gm.

TABLE IV

GROWTH (IN MG.) OF *Penicillium Camembertii* ON CZAPEK'S SOLUTION CONTAINING A 2 PER CENT SOLUTION OF LEVULOSE OR DEXTROSE

Levulose	Acidity after fungus was filtered off (expressed in cc. of N/10 alkali used)	Dextrose	Acidity after fungus was filtered off (expressed in cc. of N/10 alkali used)
105	0.3	146	0.3
159	0.3	119	0.3
143	0.3	104	0.3
115	Neutral	123	Neutral
174	Neutral	100	Neutral
158	Neutral	107	Neutral
170	Neutral	74	Neutral
143	Neutral	97	Neutral
129	Neutral		
146	Neutral		
Average: 144±4.8		109±5	

of fungus was produced and no acid formed. On the other hand, if 1.5 gm. of ammonium tartrate or citrate or potassium tartrate replaced the citric or tartrate acids, not over 0.06 gm. of fungus was produced, while as much as 0.7 per cent of oxalic acid, estimated as the calcium salt, was produced. RAISTRICK and CLARK (26) found that the four carbon dibasic acids (succinic, fumaric, malic, and tartaric) gave a good growth of *A. niger* and good yields of oxalic acid, while the four carbon monobasic acids (butyric, isobutyric, Beta-hydroxybutyric, and hydro-isobutyric) allowed neither growth nor the production of any acid. The three carbon acids (lactic, pyruvic, glyceric, malonic, and propionic) induced a good

growth of the fungus, but there was no oxalic acid produced. Acetic acid gave a good production of acid and also a good growth of the fungus. Glycolic and glyoxylic gave a fairly good growth of *Aspergillus*, but there was no oxalic acid formed. Formic acid was utilized by the fungus and a good growth resulted, but no oxalic acid was formed. This evidence illustrates how different foods affect the growth of a plant and the metabolic products it produces. It also suggests that the intermediary metabolism of plants and animals may be quite similar.

It has been noted that if *Penicillium* is grown on culture solutions similar to the one on which *Aspergillus* was grown, no oxalic acid is produced, but the solution becomes alkaline, due to the production of ammonia. MATSUMATO (**21**) noted that *Rhizoctonia* collected from widely separated geographical stations always produced acid, and the acid and growth were proportional. The writer found that when *P. Camembertii* was grown on solutions which were originally acid, these solutions became neutral or slightly alkaline.

EXPERIMENT 5.—This experiment differs from the preceding experiments in that a 3 per cent solution of the sugars was used. The results are given in table V.

TABLE V

GROWTH (IN MG.) OF *Penicillium Camembertii* ON CZAPEK'S SOLUTION CONTAINING 3 PER CENT DEXTROSE OR LEVULOSE; ACIDITY OF CULTURE SOLUTIONS WAS EQUAL TO 0.9 CC. OF N/10 ALKALI IN CASE OF LEVULOSE AND 0.8 IN CASE OF DEXTROSE

Levulose	Acidity after fungus was filtered off	Dextrose	Acidity after fungus was filtered off
331	0.2	362	0.1
334	0.1	250	0.2
300	0.2	268	0.1
		261	0.2
Average: 322		287	

EXPERIMENT 6.—In this experiment 5 per cent of either dextrose or levulose was added to Czapek's solution. The results are given in table VI. It was found that *Penicillium* produced a greater

dry weight on levulose, but the largest single culture was produced on dextrose.

TABLE VI

GROWTH (IN MG.) OF *Penicillium Camembertii* GROWN ON CZAPEK'S SOLUTION CONTAINING 5 PER CENT SOLUTION OF EITHER DEXTROSE OR LEVULOSE

Levulose	Dextrose
978	762
1042	676
1033	664
90	847
1058	772
953	915
1006	676
883	705
792	776
1085	641
1010	1180
Average: 1039	791

EXPERIMENT 7.—*Aspergillus niger* instead of *Penicillium* was used in this experiment. The results are given in table VII.

TABLE VII

DRY WEIGHT (IN MG.) OF *Aspergillus niger* GROWN SIXTY-ONE DAYS ON CZAPEK'S SOLUTION CONTAINING 2 PER CENT SOLUTION OF EITHER DEXTROSE, LEVULOSE, SUCROSE, OR MALTOSE; TEMPERATURE 20° C.

Dextrose	Levulose	Sucrose	Maltose
303	275	201	279
300	234	374	308
307	295	347	219
295	221	468	171
297	270	270	266
300	. . . . .	314	232
240	. . . . .	. . . . .	217
250	. . . . .	. . . . .	. . . . .
Average: 275	259	322	242

EXPERIMENT 8.—This experiment is a repetition of experiment 7. The results are given in table VIII. It will be noticed that the dry weights of the fungus in table VIII are twice those given in table VII. The writer is unable to explain this discrepancy. In

experiment 7, 250 cc. Erlenmeyer flasks were used, while 250 cc. globe flasks were used in experiment 8. It is possible that the mechanical hindrance of the small growing area in the Erlenmeyer

TABLE VIII

DRY WEIGHT (IN MG.) OF *Aspergillus niger* GROWN SIXTY DAYS ON CZAPEK'S SOLUTION CONTAINING 2 PER CENT SOLUTION OF EITHER DEXTROSE, LEVULOSE, SUCROSE, OR MALTOSE; TEMPERATURE 20° C.

Dextrose	Levulose	Sucrose	Maltose
529	554	503	559
539	526	620	631
534	556	622	570
535	607	479	531
550	550	412	571
517	565	591	548
.....	550	569	542
.....	565	.....	495
.....	.....	.....	495
Average: 534	559	557	549

might stop growth, and yet allow respiration. As Currie points out, *Aspergillus* folds to such an extent that it is doubtful whether mechanical resistance would be a factor. KUNSTMAN (**17**) has shown that both *Aspergillus niger* and *Penicillium glaucum* vary greatly when grown on culture solutions of the same composition containing sugar, and incubated under identical conditions.

EXPERIMENT 9.—The object of this experiment was to determine the difference in the rate, if any, in the absorption of sugars. This experiment was also carried out in 250 cc. Erlenmeyer flasks, and here the weights are not equal to those in experiment 8. The results are given in table IX.

TABLE IX

EFFECT OF 2 PER CENT SUCROSE, DEXTROSE, LEVULOSE, AND MALTOSE IN CZAPEK'S SOLUTION ON DRY WEIGHT (IN MG.) OF *Aspergillus niger;* TEMPERATURE 20° C.; STARTED MAY 8, 1920

Date of testing	Dextrose	Sugar used (mg.)	Levulose	Sugar used (mg.)	Sucrose	Sugar used (mg.)	Maltose	Sugar used (mg.)
May 22	083.9	1350.7	94.6	1092.6	127.9	657.5	84.0	865.7
May 26	304.9	1855.1	302.0	1806.3	316.9	.....	352.6	1221.0
May 28	354.2	1231.4	348.4	1892.3	.....	.....	382.5	1892.3
Average	247.6	.....	248.3	.....	273.0	.....	273.0	.....

EXPERIMENT 10.—In this experiment no quantitative sugar determinations were made, but the time of the disappearance of the sugars was noted. The results are given in table X.

TABLE X*

GROWTH OF *Aspergillus niger* (IN MG.) ON CZAPEK'S SOLUTION CONTAINING EITHER 2 PER CENT DEXTROSE, LEVULOSE, SUCROSE, OR MALTOSE; TEMPERATURE 20° C.; STARTED MAY 8, 1920

Date of harvest	Dextrose	Levulose	Sucrose	Maltose
May 10.....	171	182	155	.....
May 12.....	.....	434	471	364
May 15.....	649	360	.....	646
May 19.....	536	558	526	740
May 22.....	639	656	605	546
May 24.....	695	740	541	739
.....	636	602	.....	631
.....	569	.....	.....	473
.....	.....	.....	.....	724
Average....	557	504	460	608

* The dextrose and levulose disappeared from the solutions in fourteen days, and in sixteen days the sucrose had been absorbed. There was a trace of maltose left at the end of the experiment.

EXPERIMENT 11.—PETERSON and FRED (**23, 24**), who worked with certain pentose fermenting bacteria, found that acetic acid, lactic acid, and manitol were produced when these organisms were grown in culture solutions containing different sugars. Manitol was produced only during the fermentation of levulose. The growth of the organisms was very rapid while the sugars were being acted upon, but when the alcohol and acids were being acted upon growth was very slow. The writer's experience with *Aspergillus* indicates that its growth ceases when the sugars have been utilized, and if metabolic products are formed they are not used by this fungus.

The preceding experiments were all kept at 20°C. In order to determine whether a more rapid utilization of the sugars would influence the dry weight produced, an experiment was conducted at 30° C., the results of which are given in table XI. MOLLIARD (**22**) seems to think that *Sterigmatocystis niger* produces a greater dry weight on levulose, partly because it is utilized more slowly.

The amount of dextrose and sucrose in the original solutions was 2.3 gm., the solution containing levulose had 2.1 gm., while the flasks with maltose had 2.5 gm. At 30° *Aspergillus* absorbed the sugars in six days, whereas it took fourteen to sixteen days for the

TABLE XI

EFFECT OF EITHER SUCROSE, DEXTROSE, LEVULOSE, OR MALTOSE ON DRY WEIGHT (IN MG.) OF *Aspergillus niger*; TEMPERATURE 30° C.; STARTED MAY 10, 1920

Date of testing	Dextrose	Sugar used (mg.)	Levulose	Sugar used (mg.)	Sucrose	Maltose	Sugar used (mg.)
May 12.....	369	600.8	.....	.....	.....	428	681.6
May 14.....	694	1600.9	588	1816.8	791	647	.....
May 16.....	849	1490.9	694	1988.8	.....	562	1330.0
May 18.....	649	.....	713	.....	674	780	.....
.....	.....	.....	790	.....	711	631	.....
.....	.....	.....	718	.....	698	697	.....
.....	.....	.....	615	.....	748	654	.....
.....	.....	.....	621	.....	.....	.....	.....
.....	.....	.....	624	.....	.....	.....	.....
Average.....	656	.....	670	.....	724	628	.....

fungus to absorb the sugars at 20°. The culture in experiment 7 showed well developed mycelia at the end of the second day, while the fungus required four to five days to show the same stage of development in experiment 6. The dry weights were about the same in both cases. The temperature affected the rate, but not the amount of growth. The maximum dry weight was produced at the time of the disappearance of the sugars. This indicates that no metabolic products were produced which would induce further growth. If they were produced, they were not utilized by *Aspergillus*.

EXPERIMENT 12.—This experiment is a repetition of the preceding experiments. The results are given in table XII.

TABLE XII

DRY WEIGHT (IN MG.) OF *Aspergillus niger* PRODUCED ON CZAPEK'S SOLUTION CONTAINING 1 PER CENT LEVULOSE OR DEXTROSE

Levulose	Dextrose
262	280
272	277
261	144
244	252
255	278
273	215
259	234
297	250
270	281
261	252
254	255
Average: 273	247

EXPERIMENT 13.—In this experiment 7.5 gm. of dextrose and 7.350 gm. of levulose were used. The results are given in table

TABLE XIII

ACIDITY PRODUCED IN *Aspergillus niger*; 7 PER CENT SOLUTION OF SUGARS USED; EXPERIMENT LASTED 45 DAYS

Dextrose	Acidity	Sugar left (mg.)	Levulose	Acidity	Sugar left (mg.)
1633	6.6	274.8	1340	4.7	271.0
1338	6.6	200.5	1397	4.7	220.8
1254	5.2	65.5	1495	4.7	146.5
1233	5.2	65.5	1538	4.7	146.5
1202	3.1	Trace	1518	5.3	Trace
1280	3.1	0	1492	6.7	Trace
1208	3.8	0	1388	6.0	24.8
1347	4.0	0	1509	6.0	0
1275	3.0	0	1024	3.5	0
Average: 1315±28			1534±36		

XIII. Although the acidity in the culture solutions was rather high when the experiment was terminated, it was gradually decreasing, and would probably have disappeared if the experiment had been continued for a couple of weeks longer. BRENNER (3) has found that there is a decrease in dry weight in *A. niger* after the seventh day. The writer did not find this to be true, in spite of the fact that some of the experiments lasted over a month. In nearly all physiological work with fungi, *A. niger* or some form of *Penicillium* has been used, so the writer decided to try several of the forms which had not been so generally used.

EXPERIMENT 14.—In this experiment an unknown species of *Fusarium* was used. The results are given in table XIV.

TABLE XIV

GROWTH (IN MG.) OF *Fusarium* SP. ON CZAPEK'S SOLUTION CONTAINING EITHER 1.5 GM. DEXTROSE OR 1.7 GM. LEVULOSE; EXPERIMENT LASTED SIX WEEKS

Dextrose	Date of harvest	Sugar left (mg.)	Reaction	Levulose	Date of harvest	Sugar left (mg.)	Reaction
301	July 29	.....	0.4 alkali	335	July 23	47.5	0.3 acid
479	August 1	24.4	0.4 alkali	384	August 1	28.7	Neutral
563	August 2	.....	Neutral	336	August 2	6.6	Neutral
310	August 2	.....	0.2 alkali	415	August 8	Trace	0.2 alkali
441	August 7	.....	0.1 alkali	328	August 8	Trace	0.2 alkali
348	August 7	.....	Neutral	355	August 8	Trace	0.2 alkali
298	August 8	.....	0.1 alkali	.....	.....	.....	.....
265	August 8	.....	0.1 alkali	.....	.....	.....	.....
360	August 8	.....	0.1 alkali	.....	.....	.....	.....
Average: 374				364			

EXPERIMENT 15.—*Cylindrocladium* was used in this experiment. This fungus causes the crown canker of the rose. The results are given in table XV. The exact acidity or alkalinity could not be

TABLE XV

GROWTH (IN MG.) OF *Cylindrocladium scoparium* ON EITHER 1.8 GM. DEXTROSE OR 1.75 GM. LEVULOSE; EXPERIMENT LASTED 45 DAYS

Dextrose	Sugar left	Reaction	Levulose	Sugar left (mg.)	Reaction
474	Trace	Alkali	373	44.7	Acid
530		Alkali	484	Trace	Acid
595		Alkali	498	Trace	Neutral
507		Alkali	466	Trace	Acid
407		Alkali	407	0	Acid
353		Alkali	538	0	Neutral
433		Alkali	500	0	Acid
Average: 472			466		

secured, as the color produced in the culture solution by the growth of this fungus made the securing of an exact end point impossible.

Discussion

A survey of the literature shows that no one sugar is the best source of carbon and energy for all micro-organisms, although it is probable that most micro-organisms, excluding algae, can use most sugars to some extent. KENDALL and YOSHIDA (**16**) show that *B. proteus* does not use fructose or mannose, nor does *B. mesentericus* use mannose, galactose, lactose, or sucrose. FRED, PETERSON, and ANDERSON (**10**), and FRED, PETERSON, and DAVENPORT (**11**) have found that *Lactobacillus pentoceticus* forms manitol, acetic acid, and CO_2 from fructose; while from glucose ethyl alcohol, lactic acid, and CO_2 are formed. From the pentose sugars they found that this organism formed very little CO_2, but from the hexoses considerable CO_2 was formed. The actual amount of growth from these sugars was not stated, but it probably varied considerably. HARDIN and WALPOLE (**12**) found that *Bacillus lactis aerogenes* produced twice as much alcohol from manitol as from glucose. They attribute this to the presence of the terminal CH_2 (OH) groups, which occur twice in manitol and once in dextrose. The amount of growth of the bacillus would probably be more on dextrose than on manitol, owing to inhibition caused by the alcohol.

The composition of the synthetic media may be a factor in the utilization of sugars. It has been claimed by some that peptone and glucose is the best medium for most fungi. CZAPEK obtained best results with amino acids and glucose when growing *Aspergillus*. FISCHER (9) found that *Bacillus coli*, *B. subtilis*, and *B. pyocyamis* could use nitrate plus glucose, but on substituting glycerine for glucose, *B. pyocyamis* alone grew. LAURENT (18) found that *Alternaria tenuis*, *Mucor racemosus*, and *Aspergillus glaucus* grew well on solutions containing nitrates, but that *A. niger* thrived better when ammonia was the source of nitrogen. DOX (8) found that *Aspergillus* and *Penicillium* grew well on Czapek's solution in which sodium nitrate is the source of nitrogen and cane sugar is the source of carbon. YOUNG (31) used ammonium nitrate as a source of nitrogen, and cane sugar as a source of carbon, and secured excellent growth with *A. niger*.

Recently WEIMAR and HARTER (29), working with *Fusarium acuminatum*, *Diplodia tubericola*, *Rhizopus tritici*, *Mucor racemosus*, *Sclerotium bataticola*, *Penicillium* sp., *Botrytis cinerea*, and *Sphaeronema fimbriatum*, found great variation in the amount of dry material produced at the same concentration of glucose. The optimum was not the same for each fungus. The organisms varied in the amount of glucose necessary to produce one gram of dry weight; also the amount of glucose required to produce one gram of dry weight of the same fungus differed with the concentration of the solution.

Before the question can be answered as to what sugar is the best tissue former for any organism, such factors as the metabolic products formed in the utilization of these sugars, the character of the synthetic media, the concentration of the sugar, the temperature and perhaps hydrogen-ion concentration, and many other contributing conditions will need repeated and thorough study.

There seems to be very little evidence in the literature other than that advanced by LINDET and MOLLIARD, which indicates that dextrose is more rapidly fermented by micro-organisms than levulose. The table published by KENDALL and YOSHIDA shows that *B. proteus* will ferment glucose but not fructose. All the other seven organisms fermented both sugars, and it is quite possible

that *B. proteus* could also have acted on levulose under proper conditions. MAIGE and NICOLAS (**20**) believe that increases in the rate of respiration by sugars follow the order of their penetrability. If further investigations prove this to be true, it will be a possible explanation of the results obtained by MOLLIARD.

There is evidence in the literature, however, which points to the possibility that levulose is more rapidly used in respiration and fermentation than dextrose. HARDEN and YOUNG (**13**), using the juice from top yeast, found that levulose was slightly more rapidly fermented, and gave a somewhat larger total of carbon dioxide than dextrose. HARDEN also pointed out that, in the presence of phosphates, levulose is much more rapidly fermented than dextrose or mannose, although he also states that different samples of yeast behave quite differently toward levulose and dextrose. WALPOLE (**28**) found that *B. lactis aerogenes* decomposes glucose and fructose in a similar manner. MATSUMATO (**21**) found that *Rhizoctonia* acted on glucose and fructose with the same ease. BUCHNER and RAPP (**4**) found that both sugars were used identically by yeast. ARMSTRONG (**1**) found that twenty typical yeasts fermented mannose, levulose, and dextrose alike. WILSON (**30**) found that fructose tends to stimulate nodule production. This stimulus must be on the host plant, as PRUCHA (**25**) gives data which indicate that *B. radicicola* does not readily utilize fructose. Recently FRED, PETERSON, and DAVENPORT (**11**) have shown that a pentose destroying bacterium, *Lactobacillus pentoacetus*, ferments levulose more rapidly than it does dextrose, galactose, or mannose. This organism is able to ferment xylose and arabinose, especially the former. HAWKINS (**14**) has shown that *Glomerella cingulata* and *Penicillium* can utilize arabinose and xylose as a source of carbon.

There is a possibility that certain organisms may utilize levulose only or dextrose only. This is shown by SWARTZ (**27**), who presents a table of twelve different algae, of which number all but one contained levulose, seven contained both levulose and dextrose, while five contained no dextrose. In another table twelve cryptogams are tested, four of which contained dextrose and none contained levulose. One is inclined to be skeptical of the data in the second table, where eight species out of the twelve contained neither

dextrose or levulose, when both sugars are so universally present in growing plants.

It is evident that our information on the organic nutrition of micro-organisms is very meager. It has been shown that they are able to utilize disaccharides, hexoses, pentoses, alcohols, organic acids, and other organic compounds as sources of carbon, but the intermediary metabolism of any of these compounds remains very much of a mystery. From a survey of the literature, however, and from the data presented in this paper, the writer feels safe in concluding that the data at hand do not indicate, in the case of the micro-organisms employed in this investigation, that either glucose or fructose is a superior tissue former. Furthermore, if either sugar is more readily used in respiration, a survey of the literature indicates that it is fructose rather than glucose. Since the metabolism of so few micro-organisms has been investigated, however, we cannot make any definite conclusions as to which sugar is more used by them in respiration.

It is concluded from the data presented in this paper, and under the conditions of the experiments, that the organisms studied utilize glucose and fructose somewhat similarly, but that for *Aspergillus* and *Penicillium* fructose seems more favorable.

The writer is greatly indebted to Professor LEWIS KNUDSON for his many helpful suggestions, and for his constructive criticisms during the course of this investigation and the preparation of this manuscript.

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FURTHER EXPERIMENTS ON GROWTH OF EXCISED ROOT TIPS UNDER STERILE CONDITIONS[1]

WM. J. ROBBINS AND W. E. MANEVAL

(WITH THREE FIGURES)

Introduction

In previous papers[2] by one of the writers a method was reported for growing the isolated root tips or stem tips of higher plants under sterile conditions. Experiments were also described which showed that in the dark the isolated root tips of corn were limited in the amount of growth they would make in a modified Pfeffer's solution containing 2 per cent glucose. The addition of peptone or autolized yeast to the solution was found to permit growth to continue for a longer period. The cessation of growth in the modified Pfeffer's solution containing glucose might be ascribed to an injurious action of the solution, due to the presence of a toxic substance in the water or among the salts or sugar used, to an unbalanced condition in the modified Pfeffer's solution, to a deficiency of oxygen in the solution culture, or to a deficiency of some substance or substances essential for growth which are not found in the salts and sugar used, but which are supplied at least partially by the peptone and autolized yeast. Conversely, the beneficial effects of peptone or autolized yeast may be due to the correction of the injurious or unbalanced condition of the solution, or to the supplying of necessary nutrient material.

While glucose, the mineral salts of Pfeffer's solution, water, and oxygen include all the materials commonly assumed necessary for the growth of higher plants, the experiments referred to seemed to

[1] Published with the permission of the Director of the Agricultural Experiment Station, University of Missouri.

[2] ROBBINS, WM. J., Cultivation of excised root tips and stem tips under sterile conditions. BOT. GAZ. **73**: 376–390. 1922.

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indicate that they were insufficient for the growth of isolated root tips. Other investigators, including SACHS, HABERLANDT, HANNIG, BOTTOMLEY, WEBER, BACHMAN, LOEB, MOCKERIDGE, FULMER, WILLIAMS and SHERMAN, WILLIAMS, and others have attacked somewhat the same problem by other methods, and have decided either for or against the assumption that some substance or substances in addition to those just enumerated are necessary for the growth of plants or parts of plants. These investigations have sought for experimental data to prove or to disprove the hypothesis indicated, hoping either to succeed eventually in cultivating excised root tips continuously in a nutrient medium containing nothing but carbohydrate, water, oxygen, and the salts of Pfeffer's solution or their equivalent; or else to discover the additional material or materials which must be present for their continued growth and the source from which they come when the root is attached to the top of the plant. The present paper is the record of further experiments upon the growth of isolated root tips in the dark in sterile solution cultures.

The method used in cultivating the isolated root tips is essentially that reported earlier. Grains or seeds were sterilized, usually by Wilson's calcium hypochlorite method, and germinated on sterile agar in a Petri dish. After germination, a centimeter or more of the root tip was cut off with a sterile scalpel, and by means of a sterile wire loop transferred to the sterile medium. A single root tip was grown in 50 cc. of solution in a 150 cc. Erlenmeyer flask of Pyrex glass. The mineral nutrient solution used was a modified Pfeffer's solution containing:

	gm.		gm
$Ca(NO_3)_2$	2.0	KH_2PO_4	0.5
KNO_3	0.5	KCl	0.25
$MgSO_4$	0.5	$FeCl_3$	0.005

Distilled water 6000 cc.

Effect of different concentrations of glucose

The root tips of Longfellow flint corn were grown in the dark at room temperature in the modified Pfeffer's solution six times the strength here given, to which various concentrations of glucose were added. The experiment was begun June 11 and completed July 5,

1920. The period of growth, therefore, was twenty-four days. As can be noted from the data in table I and the curves in fig. 1,

TABLE I

EFFECT OF CONCENTRATION OF GLUCOSE UPON GROWTH OF EXCISED CORN ROOT TIPS

Addition to Pfeffer's solution	No. of roots	Average original length (cm.)	Average gain in length (cm.)	Average no. of secondary roots	Dry weight per 10 roots (gm.)	P_H at beginning	Average P_H at end
None	10	1.55	0.46	0.0	0.0070	4.75	4.99
Dextrose 0.2 per cent.	10	1.49	9.73	4.3	0.0200	4.7	5.09
Dextrose 0.5 per cent.	8	1.73	13.07	26.0	0.0275	4.7	5.41
Dextrose 1.0 per cent.	10	1.70	14.70	51.6	0.0500	4.7	5.67
Dextrose 2.0 per cent.	9	1.61	20.44	134.9	0.1570	4.65	5.81
Dextrose 4.0 per cent.	10	1.70	23.20	146.6	0.3040	4.45	6.10
Dextrose 6.0 per cent.	9	1.69	18.44	104.0	0.8030	4.30	7.17

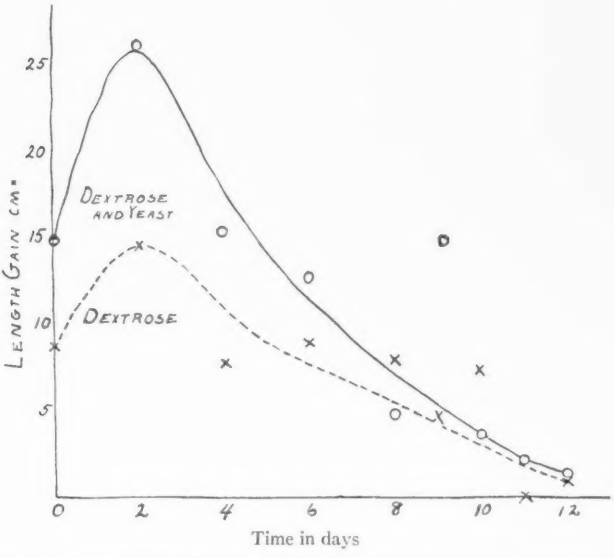

FIG. 1.—Average gain in length (solid line) and secondary root production (dotted line) of corn root tips in Pfeffer's solution plus different concentrations of dextrose.

the maximum increase in length and production of secondary roots occurred in 4 per cent sugar and the maximum dry weight in 6 per cent sugar. Most of the roots in the 6 per cent sugar were quite

abnormal at the end of the experiment, being brittle and swollen. Many were also somewhat abnormal in the 4 per cent concentration. The abnormal roots probably account for part of the increases in dry weight noted in these two concentrations of sugar, as abnormal roots such as described always show a higher dry weight than normal roots grown under the same conditions. The decreased growth in the 0.2 per cent sugar was not due to an exhaustion of the

FIG. 2.—Excised corn root tips grown twenty-four days in dark at room temperature: from left to right, in modified Pfeffer's solution, in same solution plus 0.2, 0.5, 1, 2, 4, and 6 per cent dextrose.

sugar, as a test with Fehling's solution at the end of the experiment showed sugar present. The H-ion concentration of the solutions was determined by Gillespie's colorimetric method at the beginning and at the end of the experiment, and the average results are given in table I. It can be noted there that the addition of the sugar depressed the P_H of the solution, the Pfeffer's solution without sugar showing a P_H of 4.75, and the solution to which 6 per cent sugar was added a P_H of 4.3. In all cases the solution was made

more alkaline by the presence of the root tips, and the greater the final dry weight of the root tips the greater the change in the reaction of the solution. A photograph of the best roots in the check and in each concentration of sugar is shown in fig. 2. The maximum gain was that of a root tip in the 4 per cent sugar, which was originally 1.7 cm. long and which gained 38.8 cm. and produced 276 secondary roots.

The fact that the root tips grow more in the higher concentrations of sugar does not necessarily mean that they will grow for a longer period of time. This experiment was repeated with 0.5, 2, 4, and 6 per cent dextrose in the 1*x* Pfeffer's solution. The original root tips were grown for two weeks, and at the end of that time their tips were severed and transferred to fresh solutions. This was repeated at the end of two weeks. None of the roots in 0.5 per cent sugar made any growth in the third period. Those in 2, 4, and 6 per cent sugar made a gain of 1–1.5 cm. in the third period. The difference between the roots in the three higher concentrations of sugar was slight in the third period. This would seem to indicate that in a 2 per cent dextrose solution some factor other than the concentration of the sugar limits the growth upon continued transfer of root tip tissue. It is also of interest that increasing the concentration of the salts used six times, as was done in the first experiment, did not affect the growth of the root tips noticeably.

Relation between original length of root tips and amount of growth

It was thought that some information regarding the relation between the amount of material which the root tip carries with it from the seed, and the amount of growth, could be obtained if the growths made by root tips of various original lengths were compared. Using Longfellow flint corn, groups of excised root tips averaging in length 0.35, 0.68, 1.06, 2.06, 4.04, and 6 cm. were grown in the dark in the 6*x* Pfeffer's solution plus 2 per cent glucose. The experiment was begun June 26 and concluded August 16, 1920. After the original excised root tips had grown seventeen days their tips were severed and transferred to fresh nutrient solutions, where

they were allowed to grow for thirty-four days. The data are summarized in table II. It can be noted there that the root tips averaging 0.35 cm. in length made almost as much growth, and produced almost as many secondary roots in the first and second periods as did those which had an average original length of 6 cm. The dry weight of the longer roots was greater in the first period, due possibly to the weight of the original root tips used; but in the second period the dry weight of the tips coming from the roots

TABLE II

GROWTH OF EXCISED CORN ROOT TIPS OF DIFFERENT ORIGINAL LENGTHS FOR TWO SUCCESSIVE PERIODS IN DARK IN PFEFFER'S SOLUTION PLUS 2 PER CENT GLUCOSE

No. of roots	Average original length (cm.)	Average gain in length (cm.)	Average no. of side roots	Dry weight per 10 roots (gm.)	Initial P_H	Final P_H
	June 26–July 13					
6	0.35	17.6	95	0.1550	4.5	5.87
6	0.68	14.8	87	0.1150	4.5	5.88
6	1.06	13.0	79	0.0900	4.5	5.77
6	2.06	15.6	86	0.0900	4.5	5.86
9	4.04	15.3	83	0.1750	4.5	6.04
6	6.00	18.9	24	0.2280	4.5	6.03
	July 13–August 16					
5	1.56	4.04	14	0.0240	.....	.....
6	1.55	4.30	8	0.0120	.....	.....
6	1.55	1.75	5	0.0050	.....	.....
3	1.60	2.8	14	0.0060	.....	.....
7	1.61	3.54	11.4	0.0150	.....	.....
4	1.50	4.80	15	0.0220	.....	.....

of shortest original length was as great as the dry weight of those from the roots having the greatest original length. The greatest gain for individual roots in the first period was that of root no. 3, originally 0.2 cm. long. This root gained 28.3 cm. and produced 128 secondary roots. In the second period it gained 7 cm. and produced 10 secondary roots. The next best gain was that of root no. 27, which was originally 4.2 cm. long and which gained 23.2 cm. with 145 secondary roots in the first period. If there is something in addition to the salts of Pfeffer's solution,

oxygen, water, and glucose necessary for root growth, and which is present in the original root tip when removed from the grain, most of it must be located in the last 2 mm. of the root tip. Whether there is any significance in the decreased growth shown by the root tips of intermediate length, as compared with those of shortest and longest original length, cannot be said.

The H-ion concentrations of the solutions at the beginning and end of the first period were also determined, and are given in table II. The original P_H of the solutions before growth was 4.5, and the growth of the roots made the solutions more alkaline. Again in general the greater the final dry weight the greater was the change in reaction.

Effect of extracts of corn grains and seedlings on growth of excised root tips

In a previous paper by one of the writers it was pointed out that the addition of extract of corn seedlings had no beneficial effect upon the growth of the corn roots, but was somewhat harmful at the concentrations used. It was thought that by using more dilute extracts a beneficial effect might be obtained. Additional experiments were performed, therefore, in which extracts of whole corn grains, 4-day old seedlings, or the endosperm alone were used. A corn grain was sterilized intermittently in 50 cc. of distilled water, and 1, 5, or 10 cc. of the infusion so made was added to 50 cc. of the Pfeffer's solution with 2 per cent glucose. In addition, the same solution was used to which one-half of a Canada field pea was added before sterilization. The results of the experiment showed that 10 cc. of the extract of the corn grain were distinctly injurious, while 1 cc. and 5 cc. both showed some beneficial effects on the growth. This was particularly true of the root tips which grew in the solutions to which 1 cc. of the extract of the corn grains was added. The root tips in this case made a greater growth than the check in the third period, and three out of five still continued to grow in the fourth period. The Canada field pea was apparently without effect. The results secured with the extract of the corn grain were considerably less favorable than those with the yeast extracts.

In the experiments with extracts of the endosperm or seedling plants, the embryos were separated from the endosperm of thirty-

nine seeds after four days' germination. The seedlings were extracted with 400 cc. of distilled water and the endosperms with 400 cc. of distilled water. The solutions were sterilized intermittently. Of the extracts prepared in this way, 1 cc., 3 cc., or 5 cc. was added to 50 cc. of the Pfeffer's solution containing 2 per cent dextrose. The roots were grown in the dark and carried through three periods of transfer. The root tips in those solutions receiving 5 cc. of the extracts showed evidences of injury which were particularly noticeable in the case of the extracts made from the endosperms. The 1 cc. and 3 cc. of the corn seedling extract seemed to produce some beneficial effects which appeared in the third period. Instead of stopping growth, as occurred in the check and in those culture solutions to which endosperm extract was added, the root tips in the solutions to which the corn seedling extract was added made an appreciable, though not marked gain.

Experiments were also performed in which an extract of red clover plants prepared as described by STEENBOCK and BOUTWELL[3] and supposed to be high in fat soluble vitamine was added to the Pfeffer's solution plus 2 per cent dextrose. Water extracts of metagen, a vitamine preparation of Parke Davis and Company, were also used. Neither of these extracts produced any beneficial results. The root tips stopped growing in the third period in the presence of these materials, as they did in the Pfeffer's solution containing dextrose alone. The total results of the experiments with the extracts of corn grains, corn endosperm, corn seedlings, Canada field peas, clover, and metagen did not encourage further investigation in that direction.

Time excised root tips remain capable of growth in solution cultures in absence of glucose

The excised root tips of Longfellow flint corn were placed in 40 cc. of modified Pfeffer's solution in 150 cc. Erlenmeyer flasks. All the flasks were then set in the dark at room temperature, and at intervals of two days 10 cc. of sterile 10 per cent dextrose were added to each of two flasks. The flasks were then allowed to stand approximately three weeks, and examined to determine whether

[3] STEENBOCK, H., and BOUTWELL, P. W., Fat soluble vitamine. VI. The extractability of the fat soluble vitamine from carrots, alfalfa, and yellow corn by fat solvents. Jour. Biol. Chem. 42:131. 1920.

growth had taken place. The results of the experiment showed that root tips which had stood in the solution for ten days before the addition of the glucose were still capable of growth, but none of those twelve days old or more produced any growth after the addition of the glucose.

TABLE III

NUMBER OF DAYS DURING WHICH EXCISED CORN ROOTS WILL REMAIN CAPABLE OF GROWTH IN DARK IN PFEFFER'S SOLUTION

No. of days preceding addition	Addition to Pfeffer's solution	No. of roots	Average gain in length (cm.)	Average no. secondary roots	Dry weight per 10 roots (gm.)	No. roots which did not grow
0	Dextrose	5	8.8	48	0.0812	0
	Dextrose+yeast	5	14.7	102	0.0816	0
2	Dextrose	5	14.5	92	0.0984	0
	Dextrose+yeast	3	26.1	113	0.0746	0
4	Dextrose	4	7.9	46	0.0340	0
	Dextrose+yeast	4	15.6	78	0.0475	0
6	Dextrose	5	9.2	23.6	0.0200	1
	Dextrose+yeast	4	12.8	29.0	0.0275	1
8	Dextrose	5	8.1	18.4	0.0188	1
	Dextrose+yeast	5	4.8	23.4	0.0240	3
9	Dextrose	5	4.6	12.0	0.0132	2
	Dextrose+yeast	4	14.7	40.0	0.0210	2
10	Dextrose	4	7.5	14.0	0.0195	1
	Dextrose+yeast	5	3.9	14.0	0.0156	2
11	Dextrose	5	0	3.6	0.0136	5
	Dextrose+yeast	5	2.2	8.2	0.0276	4
12	Dextrose	5	1.0	2.6	.....	4
	Dextrose+yeast	3	1.5	3.3	.....	2

This experiment was repeated, dextrose being added to five flasks after two, four, six, eight, nine, ten, eleven, and twelve days, and sufficient dextrose and autolized yeast to make 2 per cent of the former and 400 ppm. of the latter to five additional flasks at the same intervals. Each set of flasks was allowed to stand in the dark at room temperature for four weeks after the addition of the dextrose, before the data on the condition of the roots given in table III were taken. The figures given in table III are the averages for all roots whether they grew or not. All the root tips grew until

six days after they had been severed from the seed. The number that did not grow increased irregularly from that time, until only one out of ten of those receiving the dextrose on the eleventh day grew, and two out of eight for the twelve day series. The dry weight shows a sharp drop for the four day series, and the secondary root production for the six day series. The increase in length is not seriously affected until after the eight day series is reached. The root tips which stood two days without dextrose increased more in length and secondary root production than those which received the dextrose at once. This is shown also in fig. 3. In the first

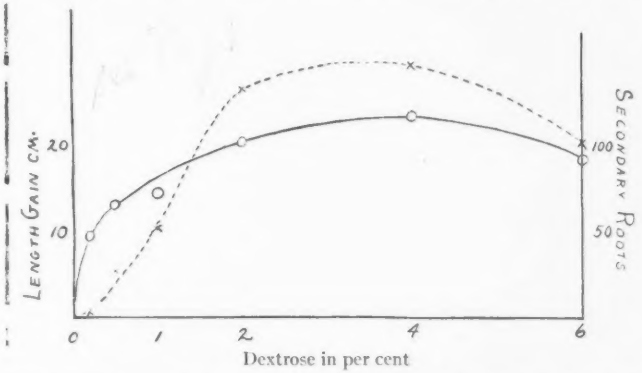

Fig. 3.—Average gain in length of root tips in Pfeffer's solution to which dextrose (dotted line) or dextrose and autolized yeast (solid line) were added at different intervals after root tips were severed from grain.

experiment the four day series showed greater increase in length and secondary root production than the two day series. The average growth of sixteen root tips for a period varying from ten to twenty-five days in Pfeffer's solution without dextrose was 0.51 cm.

Smith,[4] in determining root production from the isolated cotyledons and hypocotyls of *Cucurbita Pepo* planted in sand, found that the cotyledons and hypocotyls from seedlings ten days or more old no longer had the power to produce roots, a reduction in the ability to produce roots being noted after the seedlings were seven days old. He ascribes this failure as due to the exhaustion of the food reserves in the cotyledons.

[4] Smith, L. H., Beobachtungen über Regeneration und Wachstum an isolierten Teilen von Pflanzenembryonen. Inaug. Diss. Halle. 1907.

Effect of location of grain on ear on growth of isolated root tips

Grains were selected from the tip, middle, and butt of an ear of Longfellow corn. After germination, the root tips were transferred to the Pfeffer's solution plus 2 per cent glucose and permitted to grow in the dark for thirty-nine days. The results given in table IV

TABLE IV

RELATION BETWEEN LOCATION OF GRAIN ON EAR AND GROWTH OF EXCISED CORN ROOT TIPS

Source of grains	No. of roots	Average original length (cm.)	Average gain in length (cm.)	Dry weight per 10 roots (gm.)	Average no. of secondary roots
Tip of ear	8	1.5	13.15	0.0500	92.0
Middle	8	1.5	16.16	0.0860	91.5
Butt	7	1.5	15.25	0.0500	88

show that the root tips from grains from the middle of the ear made the greatest gain in length, and were heaviest at the end of the experiment. The difference is not striking.

Effect of sterilization of grain on growth of excised root tips

It was thought that possibly the calcium hypochlorite used in sterilization might affect the growth of the excised root tips. The growth of root tips from seeds sterilized by calcium hypochlorite was compared with that of root tips from grains sterilized by mercuric bichloride and alcohol. No consistent differences could be noted. Some sterile root tips were also secured by permitting unsterilized corn grains to germinate on sterile agar. These root tips were found to respond as did those from seeds sterilized with calcium hypochlorite.

Growth of isolated root tips of higher plants other than corn

Attempts were made to sterilize a variety of seeds and to secure sterile root tips, to see whether the excised root tips of other plants would respond as those of corn, cotton, and peas had been found to do. Beans, morning glory, white lupine, wheat, and sunflower grains or seeds were sterilized by the use of 0.1–0.5 per cent $HgCl_2$ applied for 20–40 minutes. Alfalfa, mustard, and watercress seeds

were sterilized by the use of calcium hypochlorite. Buckwheat, radish, squash, and flax seeds were not satisfactorily sterilized in the trials made; nevertheless some sterile root tips were secured in each case. The isolated root tips were grown for 23–26 days in the dark in the Pfeffer's solution plus 2 per cent glucose. The results are presented in table V. The data show that all the root tips made a fair amount of growth except radish, watercress, and white lupine. The failure of the root tips of the last plant to grow was especially interesting because the large size and strong appearance lead to the expectation of good results. Further experiments

TABLE V

GROWTH OF ISOLATED ROOT TIPS OF VARIOUS PLANTS IN DARK IN PFEFFER'S SOLUTION PLUS 2 PER CENT GLUCOSE

Plant	Period of growth in days	No. of roots used	Average gain in length (cm.)	Average no. of secondary roots
Bean	26	6	5.9	10
Radish	26	5	1.4	7
Morning glory	26	5	2.7	18
White lupine	25	2	0.45	0
Alfalfa	24	8	6.1	7
Mustard	24	6	5.9	8
Wheat	24	5	8.9	20
Sunflower	23	8	3.2	12
Flax	23	4	35.1	28
Watercress	23	4	0.4	0
Buckwheat	Some growth	.....	.....	.....
Squash	23	1	1.7	16

with the white lupine yielded but little better results. Approximately ten root tips of white lupine were placed in the Pfeffer's solution plus 2 per cent glucose, plus 2 per cent levulose, 1 per cent galactose, 2 per cent dextrose and a lupine seed, 2 per cent dextrose and 400 ppm. peptone, 2 per cent dextrose and 200 ppm. asparagine, 2 per cent glucose and 200 or 400 ppm. yeast, or 1 per cent arabinose. One root tip in the Pfeffer's solution plus 2 per cent levulose grew, and in 119 days reached the surprising length of 68 cm. with forty-five short and stubby secondary roots. The remainder showed little or no growth. We cannot explain why lupine root tips should not grow under the conditions where root tips of corn,

wheat, flax, alfalfa, mustard, and other plants do, and why the one root tip should have made such a marked growth in the solution containing levulose. Sterile root tips of lupines placed on slopes of potato dextrose agar appeared to grow somewhat more than the root tips in the solution cultures. The difference, however, was not marked.

The experiments reported here would seem to permit the following statements bearing on the ultimate cessation of growth of excised root tips in the modified Pfeffer's solution containing 2 per cent glucose. The cause is not due to too low a concentration of glucose. It is not due to injury resulting from the sterilization of the grain. If there is some material necessary for growth in addition to those supplied in the Pfeffer's solution and glucose, it must be found chiefly in the last 2 mm. of the root tip, and it cannot be successfully supplied by using water extracts of young corn seedlings, corn grains, corn endosperm, Canada field pea seeds, or by a clover extract or extract of metagen. The lupine root tips, in their failure to grow when excised, may evidence an exaggerated form of the condition which ultimately causes excised corn root tips to stop growing. If so, it is difficult to see how an accessory substance is involved, as the large lupine root tip would be expected to contain as much accessory material as the smaller corn root tip.

Summary

1. In a twenty-four day period excised corn root tips under sterile conditions make a greater gain in length, and produce more secondary roots in the dark in Pfeffer's solution containing 4 per cent dextrose, than when 0.2, 0.5, 1, 2, or 6 per cent dextrose is present.

2. When the root tip tissue of excised roots is transferred at two weeks' intervals, the gain in the dark in the third period is about the same in Pfeffer's solution containing 2–4 or 6 per cent dextrose.

3. Excised root tips of corn whose original length is 0.35 cm. grow as much, and produce almost as many secondary roots in two periods of transfer, as roots whose average original length is 6 cm.

4. Extracts of corn grains, corn seedlings, corn endosperm, Canada field peas, clover, and metagen were not found to favor the growth of excised corn root tips to any marked degree.

5. Excised root tips of corn in sterile Pfeffer's solution without dextrose may remain capable of growth for twelve days at room temperature in the dark.

6. Excised root tips from grains from the middle of the ear grew somewhat more than those from the tip or butt.

7. Excised root tips of beans, morning glory, alfalfa, mustard, wheat, sunflower, and flax were found to be capable of making considerable growth under sterile conditions in Pfeffer's solution containing 2 per cent dextrose. The results with radish, watercress, squash, and buckwheat were inconclusive.

8. The root tips of white lupine could not be grown in Pfeffer's solution containing various organic materials. One root tip of 90 grew and that to a length of 68 cm.

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ELECTRICAL CONTROL OF ORGANIC POLARITY IN THE EGG OF FUCUS

E. J. Lund

(WITH PLATE XXVII, AND TWO FIGURES)

Introduction

Organic polarity in animal and plant cells, organs, and whole organisms is one of the fundamental common properties of living material, and therefore it makes little difference whether the phenomena are considered in plant or in animal cells, the problem is probably, if not certainly, the same. This fact has long been recognized, but the problem of polarity is only a special (although in all probability primary) part of the general and more comprehensive problem of the origin and causes of symmetry in living material. An excellent treatment of certain aspects of the principle of symmetry in its relations to chemical and biological science is given by Jaeger (3).

The facts of physiology relating to growth, differentiation, and dedifferentiation have shown beyond question that the visible structure of the cell or organism is only a result of preceding physicochemical processes, in the same sense that the formation of a precipitate is the result of a chemical or physical process, which in turn is always associated with an energy change. From this fundamental fact it follows that the energy changes, or forces which determine the polar or symmetrical arrangement of the material of living cells, organs, and whole organisms must to a greater or less degree be directed forces. Such energy changes or forces must have an axial or polar orientation, corresponding in degree to the axial or polar arrangement of the material laid down as structure. The kind of information desired, therefore, is facts relating to the type, magnitude, and distribution of the associated energy changes, and not primarily a knowledge of the quality or quantity of the materials laid down as structure. The primary problem, therefore, would appear to be the nature of the forces which bring about the char-

acteristic orientation of the materials in the process of formation of the colloidal structure of cells.

It is well known that light is capable of determining the polarity of the germinating spores of many lower plants. This has been shown in the recent experiments by Miss HURD (1) on the egg of *Fucus*. Effects of mechanical pressure and centrifugation of animal eggs in relation to orientation of cell cleavage have been described by many workers (see KELLICOTT 4), but so far as the writer is aware, the following is the first successful attempt at controlling the polarity of an egg and the future body axis by means of an electric current. The object in the present paper is (1) to show that by the application of a difference of electrical potential to the egg of *Fucus*, it is possible to determine the axis of symmetry of the egg and future plant body; and (2) to give a method by means of which it has been possible to calculate the magnitude of the potential difference necessary for this orientation.

Fucus inflatus is monoecious, and consequently the eggs are fertilized at the time of shedding. The first cleavage, which is equal, begins approximately sixteen hours after the shedding of the eggs, and is completed in a few hours at room temperature. The eggs are perfectly spherical, and no axis of symmetry is evident before cleavage begins. As is well known, one of the cells of the first cleavage becomes the rhizoid or holdfast, while the other gives rise to the thallus, thus determining definitely the future polar axis of the organism.

Methods

The procedure in obtaining the eggs of *Fucus* was the same as that described by Miss HURD. Plants with mature tips were collected from midtide level near the Friday Harbor laboratory, and wrapped in moist cloths for periods varying from ten to eighteen hours, and then exposed to air for about half an hour. The tips were then removed and floated in flat dishes containing fresh sea water. Within a few minutes or half an hour the tips began to shed the eggs, which then settled down upon the bottom of the dish and could be collected and removed with a pipette. The possibility of carrying out the experiments described depends entirely upon the fact that after fertilization the *Fucus* egg fixes itself

permanently to the solid substratum (cover glass, for example) upon which it comes to rest. Permanent fixation takes place in about six to eight hours, or in some cases within a shorter time. All of the experiments with the electric current were carried out in total darkness, so that none of the results are attributable to light.[1]

The apparatus used is shown in text fig. 1, which is about one-half the actual size. *A* is a general view of the arrangement, while *B* is a longitudinal section through the arm *d* of the trough shown in *A*. The V-shaped arrangement of the two arms *c* and *d* of the trough in *A* provided for a continuous flow of a stream of sea water, whose rate of flow was regulated by the stopcocks *e* and *f*. The stopcocks were connected by siphon to the same supply of fresh sea water kept in glass jars. The level of the water in the two arms of the bath was regulated by the adjustable outflow *g*. The apparatus was made from a glass plate having three holes bored through it. The two holes at the ends of the V-shaped trough were covered with porous clay diaphragms (*x*) sealed to the glass by means of de Kotinsky cement, as shown in *B*. Into the other hole (*h*) was fitted the outflow tube *i*, in which the zinc rod electrode was held by a special support not shown in the figure. The electric current was led into the arm (*d*) of the trough from the dish of sea water (*j*), in which the other zinc electrode was supported. This is shown best in the section *B*. The sea water in the dish *j* could be changed continually if necessary, thus removing all possibility of any products of electrolysis from entering the stream of water over the eggs. The products of electrolysis at the electrode *z* were washed away by the constant stream of sea water from the two arms of the trough, thus insuring for the eggs absolute freedom from contact with any foreign chemicals. As a result of this arrangement, the electrical resistance of the arm (*d*) of the trough was relatively low, so that a relatively large adjustable resistance (*r*) could be inserted, thereby making possible a complete control of the density of the electric current through the arm of the trough (*d*). The electric current from the battery *b* (about 18 volts)

[1] The writer is greatly indebted to his assistant, Miss GLADYS HAMM, for the work connected with the preparation of the material for experiment.

was passed through an accurately calibrated milleammeter reading to 0.05 of a milleampere. The two arms of the trough provide

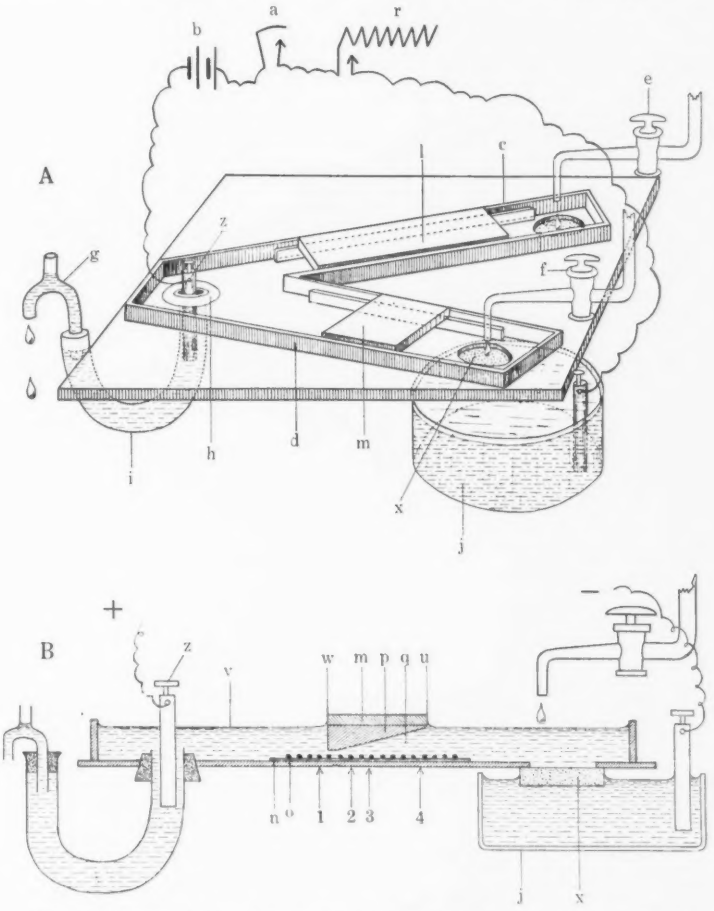

FIG. 1.—*A*, general view of apparatus; *B*, longitudinal section of arm; explanation of details in text.

for identical conditions except for the electric current passed through *d*. The trough was made from accurately fitted glass strips held together by de Kotinsky cement, and covered by glass plates

(*l*, *m*), so as to provide a definite constant cross-sectional area of the stream of sea water in which the eggs were placed. Since the total electric current flowing through the arm *d* of the bath is known from the milleammeter reading, and the cross-sectional area of the column of sea water over the eggs is constant and known, then the current density (the number of microamperes per sq. mm. cross-section of the sea water) is known. Finally, if we can determine the resistance per unit length of the column of sea water under the glass cover *m*, then of course the fall of electrical potential over any cross-section of water column can be calculated. Text fig. 1*B*, which is a longitudinal section of the arm *d*, shows the essentials of the arrangement to better advantage. The cover glass (*n*) bearing the eggs (*o*) is fixed in place on the bottom of the trough by a thin film of vaseline. To the under side of the glass cover (*m*) is fixed a glass wedge (*p*), whose width is such that it fits snugly between the sides of the arm *d* of the trough. This provides for a sharply defined wedge of sea water (*q*) whose dimensions can be determined, and therefore provides for a gradient of known density of the electric current, and therefore a gradient of known fall of electrical potential over the eggs spread upon the cover glass. The electrical resistance of this wedge of sea water of known dimensions was determined in the ordinary way, using platinum electrodes which were inserted in the positions *u* and *w* covering completely the two ends of the water wedge. To check the accuracy of this method of determining the resistance of the wedge, one of the electrodes was placed in the position *u*, while the other was placed at some definite position *v*. The resistance of the sea water column was then determined with the electrodes at these positions. The electrode at *u* was then removed to the position at *w* and the resistance again determined. The difference between these values was the resistance of the water wedge *q*. Identical values (195 ohms) for the wedge of sea water were obtained by the two procedures.

Experimentation

Several preliminary experiments were undertaken to discover the appropriate current density at which effects of the electric current upon the eggs appeared. The use of the wedge facilitated the work; for, as was found in the writer's previous work on the

electrical control of polarity in regenerating internodes of *Obelia* (6), a definite threshold of current density for orientation of the first cell cleavage exists in the *Fucus* egg.

In the first experiments the wedge was not used, but instead the current density was varied by placing the cover glasses holding the eggs upon glass squares of different thicknesses, which were then placed on the bottom of the trough under the glass covers (*m*, *l*). The results of a successful experiment, in which the appropriate current density for orientation was accidentally found in this way, is given in Plate XXVII, *a* and *b*, in which *a* is a typical field on the cover glass, the eggs upon which had been subjected to the electric current, while *b* is a field from the cover glass kept as a control in the other arm of the trough without the electric current. Other controls were also kept in finger bowls. These showed identical conditions of growth with that of the control *b*.

The photomicrographs *a* and *b* were taken seventy-one hours after seeding the eggs on the cover glasses, and seventy-two hours after shedding of the eggs by the plant tips. The electric current was turned on immediately after the eggs were placed on the cover glass, and at the same time the water current was turned on through the arm *c* of the trough, thus serving to wash the electrode *z*. The sea water in the dish *j* was changed several times during the experiment, thus insuring freedom of the eggs in *d* from chemicals, even though no water current was flowing through *d*. Since it takes about six to nine hours for the eggs to become firmly fixed, the current of sea water was not turned on in *d* until nine hours after seeding. The velocity of flow of sea water over the eggs in the arm *c* was slower than that over the eggs in arm *d*, because of the greater cross-sectional area of the water column in *c;* hence the control eggs in arm *c* were not in danger of being dislodged by the water current, even though this was started immediately after seeding the eggs upon the cover glass. This procedure permitted the eggs on the cover glass in *d* to become fixed firmly in relatively quiet water, while the electric current was being passed through the eggs from the beginning.

When the eggs in *d* had become fixed, the water current was turned on without danger of losing any of them. As time went on after seeding, the eggs became more and more securely fixed, until

at the end of about twenty-four hours the swiftest current did not seem to be able to dislodge them.

In one of the preliminary experiments eggs were placed upon two glass squares of different thicknesses. These two glass squares were then placed on the bottom of the same trough *d* and the electric current passed through. The water was turned on in the arm *c* at the beginning, as in the experiment just described. Later the water current was turned on in *d*. The result was that the eggs upon the thicker glass square (higher current density) were oriented, while those on the thinner glass square were not.

A cover glass with control eggs previously kept in sea water in a finger bowl until they had become fixed was then put into the arm *c*, and the water current allowed to flow much more swiftly through the arm *c* than over the eggs in the arm *d*. No trace of any orienting effect of the water current upon the eggs in *c* could be seen. These and other similar preliminary experiments convinced the writer that the orienting effects observed in the electric current were not due to the flow of water, nor were they due to any reaction by the egg cells to chemicals from the electrodes. In figs. *a* and *b* no difference in the amount of growth of the eggs can be seen. The eggs in the control *b* appear slightly larger than those in *a*, but this is simply due to an accidental difference in magnification. Under the microscope no difference between the two lots of eggs, except that of orientation, could be seen. These experiments, together with others giving similar results, show that it is possible to orient the first cleavage of the egg, and therefore the polarity and future body axis by an electric current.

The following experiment is reported in full because the procedure enables us to determine the quantitative relations between the current density, or electrical potential, and the effects upon the eggs. The eggs were placed on cover glasses within one hour after being shed. Some of these cover glasses were kept as controls in finger bowls containing fresh sea water. One of the covers was placed in the arm *d* of the trough under the glass wedge exactly as shown in text fig. 1*B*. The water current was turned on through the arm *c* to wash the electrode *z*, and the electric current turned on in *d* immediately after seeding the cover glass. Twelve hours after

the beginning of the experiment the water was turned on in *d*. Fourteen hours after the beginning of the experiment a cover glass containing control eggs was removed from the finger bowl and placed in the arm *c* of the trough. Forty hours after the beginning of the experiment the cover glass in *d* was removed for examination, and photomicrographs of typical microscopic fields were taken at the positions numbered 1, 2, 3, and 4 in text fig. 1*B*. These figures are correspondingly numbered 1, 2, 3, and 4 in plate XXVII. It will be seen that the eggs shown in figs. 1 and 4, corresponding to positions 1 and 4 in text fig. 1*B*, do not show any orienting effects of the electric current.

Growth in these positions was not different from growth in the control kept in the arm *c*, or those in finger bowls. In all such lots of eggs there are always some eggs which do not divide, similar to what occurs for example in echinoderm eggs, so that the few eggs showing no growth have no special significance.

Fig. 2 from position 2 shows practically 100 per cent orientation. Examination of the eggs in position 2 showed that the planes of the first cleavage were very nearly at right angles to the direction of the electric current. This fact was verified by several members of the laboratory at the time.

Fig. 3 from position 3 in text fig. 1*B* shows that there is a very distinct threshold of current density (or *PD*) at which orientation occurs. The writer believes that this may prove to be an important fact for the significance of the phenomena of orientation as a whole. Similar well defined thresholds of electrical potential have already been described for *Obelia* (**6**). On the left side of fig. 3 most of the cells are oriented, while on the right side of the same field no orientation effects are evident. The attempt was made to show the region of the threshold in the middle of the photograph, but unfortunately manipulation of the camera resulted in a slight displacement, so that the orientation threshold lies at the left of the middle of the figure.

Under the glass wedge between the positions 1 and 2 a definite inhibition (delay) in cleavage occurred. Development occurred in most of these delayed eggs after removal of the cover glass to a finger bowl with sea water. As seen from the experiments (figs. *a* and *b*), orientation by the electric current does not necessarily involve

inhibition (delay) of cleavage and growth. This is again evident in figs. 1, 3, and 4, and consequently places the fact of orientation in a definite category of what might be called normal response comparable with the response to light as found by Miss HURD (1).

Figs. *a*, 2, and 3 show that orientation of cells to the electric current is not perfect wherever the eggs lie in close groups. This may be interpreted simply as due to the fact that the electrical resistance of the group is high, and therefore the fall of electrical potential across an egg in such a group is less (that is, below the threshold value, other things being equal) than it would be if that same egg were isolated. An instance of group orientation as described by Miss HURD is shown in fig. 4. This orientation by the cell to adjacent cells undoubtedly modifies the response to the electric current, similar in a way to the interference effect of a contact stimulus upon *Paramecium* when reacting to an electric current.

Calculation of threshold value of electrical potential for orientation

Let fig. 2 represent the shape of the water wedge *q* in text fig. 1*B*. Since the total electric current (*C*) flowing through the wedge from face A_1 to face A_2 was known, and equal to 37.4 milleamperes in the experiment from which figs. 1, 2, 3, and 4 were taken, and the total resistance (*R*) of the wedge was 195 ohms, then by Ohms' law, *E*, the total fall of electrical potential between the faces A_1 and A_2, was 7.29 volts. The P.D. causing the current to flow through any given egg is the P.D. between two planes parallel to A_1 and A_2, and separated by a distance equal to the diameter of an egg. Since the diameter of the *Fucus* egg is nearly 0.08 mm., the problem becomes one of calculating the magnitude of the P.D. between any such two planes 0.08 mm. apart, at any position along the length of the wedge, as for example a_1 and a_2 in fig. 2. Since the specific resistance *K* of the sea water (in this case the resistance of a millimeter cube of sea water) is given by the equation $K = R\frac{1}{L}\frac{(A_1+A_2)}{2}$ (1), in which all the terms except *K* can be determined by direct measurement, and where $R = 195$ ohms (total resistance of water wedge); $L = 29$ mm. (total length of water wedge); and A_1 and $A_2 =$

13.5 and 159 sq. mm. respectively (areas of ends of water wedge); then $K = 580$ ohms.

The form of the equation for calculating the resistance of the wedge when K is known is therefore $R = \frac{KL2}{(A_1+A_2)}$ (2). Since in equation (2) R is the total resistance of the water wedge, and since any cross-section of this wedge is similar to the whole, for any such section of the wedge as shown in fig. 2 between the parallel planes a_1 and a_2, we have $r = \frac{580 \times 2 \times l}{a_1+a_2}$ (3); where r = resistance of wedge

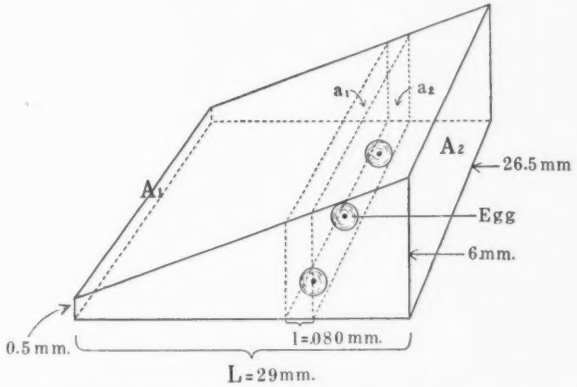

FIG. 2.—Diagram of water wedge q in fig. 1B; numbers give actual dimensions in millemeters.

section between any two planes with areas equal to a_1 and a_2, and $l = 0.08$ mm., the diameter of the egg.

The values for the areas of the planes a_1 and a_2 for each one of the positions 1, 2, 3, and 4 were determined by direct measurement from a plaster cast of the space below the glass wedge p in text fig. 1B. Since the distance between a_1 and a_2 is very small, the areas of a_1 and a_2 at any one position may be considered equal.

Applying equation (3), we can now determine r for these sections, and, since the total current is known, the P.D. between their faces, that is, the actual fall of electrical potential through an egg at these positions. Table I gives the values for the positions from which the photographs were taken. It will be seen from the table

that the threshold P.D. for orientation is 0.025 volts. This value is several times greater than the electrical potentials which are effective in producing the results described by the writer for *Obelia* (6).

The difference between these thresholds of P.D. may be due in part to the fact that the *Fucus* egg possesses a tough membrane, which may have a high resistance to the electric current. It will be clear, of course, that the density of the electric current in the egg

TABLE I

Position of photograph	$\frac{580 \times 2 \times 1}{a_1 + a_2}$	r (ohms)	P.D. across egg (volts)	Current density (microamperes per sq. mm.)
1.....	$\frac{580 \times 2 \times 0.08}{351.0}$	0.26	0.009	210
2.....	$\frac{580 \times 2 \times 0.08}{76.6}$	1.21	0.036	970
3.....	$\frac{580 \times 2 \times 0.08}{109.4}$	0.84	**0.025**	**680**
4.....	$\frac{580 \times 2 \times 0.08}{111.3}$	0.41	0.012	330

may be entirely different from that in the sea water, depending upon the ratio of the specific resistance of the egg protoplasm to that of the sea water. The last column in table I gives the densities of the electric current in the sea water at the positions of the photographs. The limits of accuracy of the method were tested by making calculations based on different sets of measurements of the water wedge. These showed, for example, that the different values for the threshold P.D. did not differ by more than 0.002 volt. Consequently it is believed that the values given lie within an error of 15 per cent of the actual values.

Discussion

Our present knowledge concerning the phenomena of orientation of the axis of symmetry of cells to an electric current is too limited to warrant any final conclusions as to its actual significance for morphogenesis, but attention may be called to certain facts related to the problem. Many free living cells orient to the electric

current by movement of the cell as a whole, but in these experiments we are confronted with a process involving orientation of cell division and growth (synthesis of protoplasm). A close similarity appears to exist between the results of INGVAR (**2**) on the oriented growth of nerve cells in tissue cultures placed in an electric field, and the growth processes described here. The main difference is that in the *Fucus* egg we have cell division superposed, so to speak, upon the growth process (synthesis of protoplasm) as such. The orientation of *Amoeba* to an electric current is also of interest, since in this case morphological polarity is a fluctuating property, and in all probability may be considered identical with fluctuating functional polarity. A series of striking cases of what appear to be transitional conditions between functional polarity and morphological polarity and their reversal, in a cell (*Bursaria*), has been described by the writer elsewhere (**7**). In these cases it is clear that a highly specialized and apparently fixed visible morphological polarity in a single cell may disappear by a process of dedifferentiation (autolysis?), part of the material of the cell reappearing with a different polarity from what it had before. The facts cited show a very close interrelation between definitely directed energy transformations and the processes of orientation of the materials laid down as morphological structure (= colloidal crystallization?) in the process of cell morphogenesis, as stated in the first part of this paper. The experiments and suggestive speculations of LILLIE (**5**) are to the point in this connection. If the physico chemical process in the cell, which determines morphological polarity, can be oriented by the application of a difference of electrical potential of external origin, does not this imply that the mechanism determining the morphological polarity possesses electrical properties?

The orientation by light presents no necessary difficulty, of course, for it is a well known fact that bioelectric currents are associated with photochemical changes, for example, plants, retina of the eye. The occurrence of constant electrical potentials in tissues and organs have long been known, for example, in epithelia, roots and shoots of seedlings, MULLER-HETTLINGEN (**10**), hydroids, MATHEWS (**9**), LUND (**8**). These electrical potentials correspond to the polarity and symmetry properties of the tissues.

From these facts the following interesting question arises. May not such electrical differences of potential present in cells and tissues act upon adjacent cells and cell groups in such a way as to bring about orientation effects such as appear in phenomena of cell correlation, where cells in contact with one another behave in morphogenesis as an ordered whole? The writer believes that such an assumption may prove a valuable guide to the investigation of the nature of the phenomena of cell correlation. Experimental facts bearing upon this question will be presented elsewhere.

Summary

1. Polarity of the egg of *Fucus* can definitely be determined by applying a difference of electrical potential to the egg. Apparently this is the first egg, among either animals or plants, the polarity of which has been controlled by means of an electric current. The response of the *Fucus* egg to the electric current in total darkness is similar to its response to light.

2. A method is given by which it is possible to measure the P.D. across a single egg which is placed in an electric current flowing in a stream of sea water of constant cross-section.

3. The magnitude of the electrical potential applied to opposite sides of an egg, which is just able to cause orientation (threshold P.D.) of the axis of symmetry of the protoplasm of the *Fucus* egg, is 0.025 ± 0.002 volts.

4. This threshold P.D. is definite and does not appear to involve any marked inhibitory effect (delay) upon growth. A P.D. of 0.036 volts applied to the egg does show an inhibitory effect (delay) upon cleavage.

5. The establishment of an electrochemical polarity of some sort may possibly be a fundamentally associated condition for the development of morphological polarity, because the physiological mechanism in the *Fucus* egg which determines morphological polarity can be controlled and directed by the application to the egg of a difference of electrical potential of external origin.

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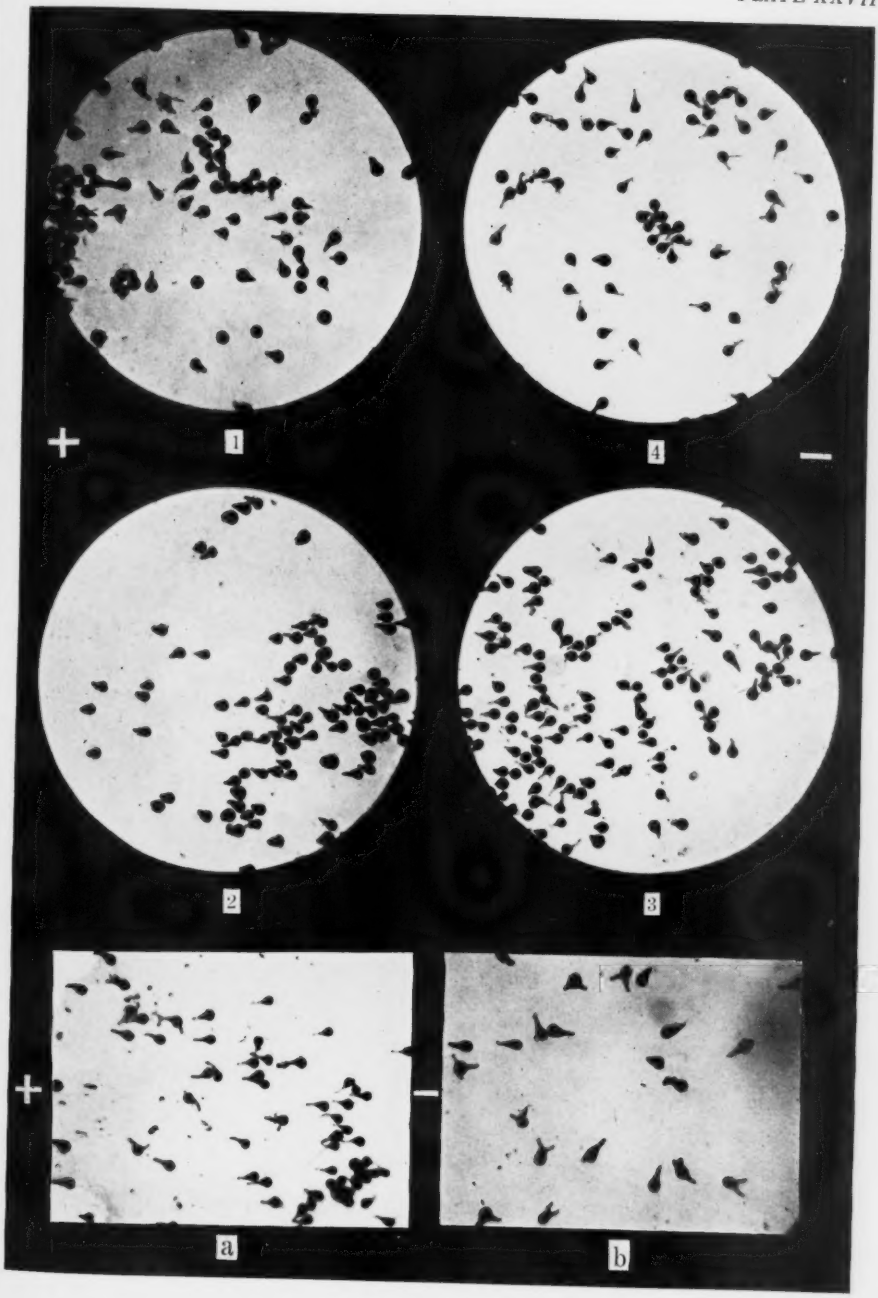

LUND on FUCUS

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GROWTH OF POLLEN TUBES IN SELF-POLLINATED APPLE FLOWERS

ISAWO NAMIKAWA

(WITH NINE FIGURES)

It is well known that certain apple varieties are self-sterile, and since this fact is of economic interest to fruit growers, numerous experiments have been conducted to determine the cause of this self-sterility. The explanations offered by different workers do not always agree.

OSTERWALDER (4) reported that in the variety Boemischer Rosenapfel sterility resulted because the pollen tube growth was insufficient to reach the ovule in self-pollinated flowers. He found that in the case of cross-pollinated flowers the pollen tubes penetrated the ovary about two days after pollination. In the same period, however, with self-pollinated flowers, the pollen tubes had grown only 2–4 mm. deep in the styles, and had formed club-shaped swellings at the tip which took the stain to an intense degree. Even after sixteen days the self-pollinated flowers showed no evidence of fertilization, or that the pollen tubes had reached the seed core.

KNIGHT (2) studied the self-sterility of the Rome Beauty. He detected no evidence that the stigmatic excretion of a variety inhibited germination and growth of pollen tubes of the same variety. He also found that the stigma offered no particular mechanical obstruction to the penetration of its pollen tubes. Instead, he found the pollen tubes grew sufficiently deep to reach the egg. KNIGHT concluded that "one important factor in self-sterility of Rome Beauty is the relatively slow rate of growth of Rome Beauty tubes in Rome Beauty stylar tissue." There is lacking, however, a definite description of histological details. It would be interesting to repeat KNIGHT's work and trace the course of the pollen tube growth, and especially to examine critically the abnormal conduct of tubes as reported by OSTERWALDER.

During the writer's stay with the Division of Pomology of the University of California, from March to July 1922, he studied histologically certain phases of the problem of self-sterility of the apple. The results obtained are not complete, but this paper is presented as a preliminary report.

Material and method

The Yellow Bellflower was the variety of apple chosen, since this is known to be self-sterile (**1, 3, 5, 6, 9**). The material was taken from an orchard near Sebastopol in Sonoma County during April 11–18, 1922. Young flowers which were promising to open within two or three days were utilized. All anthers were carefully removed from the flowers with tweezers, and the flowers covered with paper bags. Pollen of the same variety was gathered from flowers somewhat older, but with the anthers not yet opened. Two or three hours after removal and drying in open air, the anthers began to burst, and after one day dried pollen was obtainable. Germination tests with 10 per cent cane sugar solution gave about 88 per cent germination. Pollination was performed when the excrete appeared on the stigmatic surface of the emasculated flowers. At intervals up to seventy-two hours the pollinated flowers were removed and fixed in Flemming's solution. Before fixing, however, the calyces and the pubescent epidermis outside of the ovary were scraped off with a small knife. This made possible a more prompt reaction of the fixing reagent, and facilitated the cutting of the material after it was imbedded in paraffin. The material was prepared for sectioning by the usual paraffin method. In order to keep the material soft enough to cut, however, chloroform was used as a clearing agent instead of xylol. Haidenhein's iron-haematoxylin was used for staining. This method proved satisfactory for the writer's purpose. Sketches of the material were drawn from the slides by use of the camera lucida.

Results

It is generally believed that the pollen tubes of the apple penetrate the stigmatic tissue and grow downward inside the styles. The writer, however, found that this does not appear to be the

normal passage of pollen tubes. In the material taken twelve hours after pollination, most of the pollen grains had germinated, and the tubes had grown down along the outer surface of the styles. The mature and receptive styles were somewhat moist with stigmatic excrete. It appeared in some cases that moisture was derived by capillarity along the growing pollen tube, so the tube grew down within a thin film of stigmatic excrete. After reaching the base of the styles, the tubes pushed into the cavities which existed

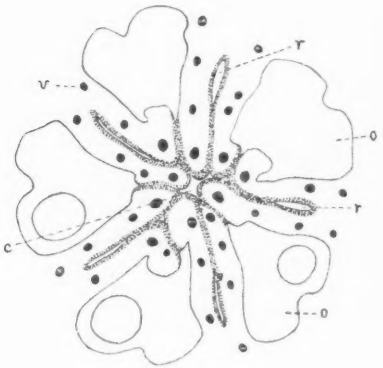

FIG. 1

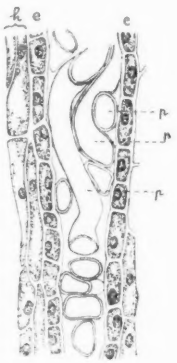

FIG. 2

FIGS. 1, 2.—Fig. 1, transverse section of middle part of ovary: *o*, ovary core; *r*, radiated cavity formed between carpels; *c*, central cavity; *v*, vascular bundle; dotted portion, conducting tissue with loose texture; fig. 2, longitudinal section of styles near insertion, with pollen tubes between them: *p*, section of pollen tube; *e*, epidermal layer of style; *h*, hypodermal layer; 400/1.

between the insertion of the styles. As shown in the cross-section of the ovary, the contact planes of carpels are not completely fused, but cavities are left radiating from the center (fig. 1*c*).

In the style there is little difference between the character of the epidermal layer and the inner tissue or hypodermal layer (fig. 2), but in the cavity there is obviously differentiated a papillary epidermal layer of cells (figs. 3, 4). Each epidermal cell has a thin membrane, rich cytoplasm, and few plastids, and presents a gland-like appearance. In fact, in the mature flower, it is apparent that the cavity is wet with a fluid having a somewhat sweet taste.

Probably this is excreted by the papillary epidermal layer of the cavity. On the other hand, the cells of the hypodermal layer are large, with thick membrane, conspicuous vacuoles, and contain an abundance of starch grains (figs. 3, 4). The cavity generally becomes narrow as it proceeds downward, and the epidermal layers of opposite sides come in contact with each other. In the upper portion of the stylar cavity the epidermis consists of a single layer, but becomes two or three cells thick as it goes deeper (fig. 5). These conducting cavities open into a central cavity, which forms an inverted funnel shape, extending to the bottom. The

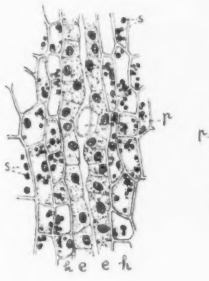

FIG. 3

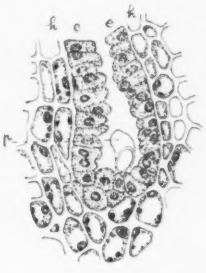

FIG. 4

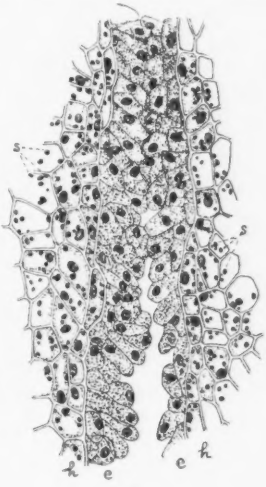

FIG. 5

FIGS. 3, 4, 5.—Fig. 3, longitudinal section of upper part of conducting cavity: *p* pollen tube; *e*, epidermal layer; *h*, hypodermal layer; *s*, starch grain; 400/1; fig. 4, arbitrary section of conducting cavity with papillary epidermal layer; 400/1; fig. 5, longitudinal section through lower part of conducting tissue; 400/1.

fusion of the epidermal layers of the conducting cavity is only partial, and in some places they are only in contact; hence the pollen tubes readily push through most of the cavity. It also appears that the pollen tubes excrete a pectose digesting enzyme (**7**). Through the activity of the pectinase, the passage of the pollen tube may be aided by dissolution of the middle lamella and the maceration of the tissue. The writer frequently observed partially and completely isolated cells adjacent to the path of the pollen tubes, although no degeneration of these cells had taken place (fig. 6).

This is the manner in which all the tubes grew down and penetrated the central cavity with their tips. In following this course, however, the pollen tubes did not always grow straight, but the direction of growth continuously curved in every way. Hence, even with properly made longitudinal sections, the tube was observed as showing many sections in different directions.

Of the flowers collected twenty-four hours after pollination, about one-half showed the pollen tubes as having reached the central cavity. With the material fixed forty-eight hours after pollination, the tubes had reached the central cavity in practically every case. During the first forty-eight hours, until the tip penetrated the central cavity, the pollen tubes grew along the outer surface of the carpels. Subsequently the tubes turned the direction of growth toward the suture of the carpel margins, where there exists a loose tissue. This loose tissue consists of cells with rich plasmatic contents, and thin cell membranes with no or few plastids, similar to the epidermal layer of the conducting tissue previously described. The pollen tubes grew through this tissue into the ovary core (fig. 7), so that at first the tubes appeared on the back of the anatropous ovule. In material fixed seventy-two hours after pollination, there were a few cases showing tubes in the ovary core, but they had not yet reached the micropyle. In most of the material the tube in the core could not be found at all.

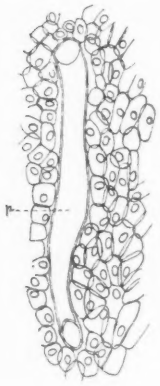

FIG. 6

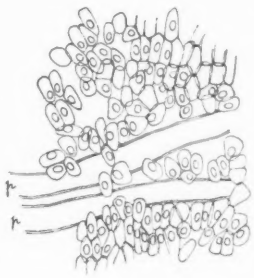

FIG. 7

FIGS. 6, 7.—Fig. 6, longitudinal section through conducting tissue, showing bit of pollen tube in it; 400/1; fig. 7, part of pollen tubes growing through loose tissue between margins of carpel into ovary core; 400/1.

For comparison, some non-treated and exposed flowers in the same stage of development as the selfed ones were fixed. In these

exposed flowers, the rate of the growth of pollen tubes was the same as with the selfed flowers. There was no definite evidence, however, that the exposed flowers were cross-pollinated. Nevertheless, it seems likely that some of the pollen reaching the stigmatic surface of these exposed flowers would be from varieties other than the Bellflower. The Bellflower trees used were closely adjacent to trees of several other apple varieties, and insects were active.

To further determine the normal growth and passage of the pollen tube, material was obtained from the Watsonville district of Santa Cruz County on May 8. At that time the blooming of the Yellow Bellflower was almost ended and only a few flowers were open. The differences between fertilized and unfertilized flowers could clearly be recognized in the case of those flowers which had bloomed somewhat earlier. The fertilized flowers were beginning to shut the calyces with hyponasty and to turn the top of the flowers upward, while the ovary had swelled a little. The unfertilized flowers did not make such changes, and the flower stalk, receptacle, and calyx had a yellowish tinge. Such flowers as appeared to be fertilized were gathered, prepared as previously described, and the growth and passage of the pollen tubes studied histologically. The behavior of the pollen tubes was essentially the same as that described for the selfed material. From these results it may be assumed that the mode of growth of the pollen tube in the selfed flowers did not show any peculiar abnormal feature. Furthermore, the growth of the pollen tubes of the selfed Bellflowers was not appreciably delayed (**4, 8**). This does not agree with the findings of OSTERWALDER (**4**) and KNIGHT (**2**).

It is of interest to note here an experiment previously performed by the writer at Hokkaido Imperial University in northern Japan. During the pollination work with apples in 1916, flowers of the Ralls (Ralls Janet) were pollinated with pollen from Jonathan. The styles of the flowers of the Ralls had previously been cut away at the point of attachment with a sharp knife, hence there was no true stigmatic excretion present. Nevertheless, at the time of pollination the cut surfaces of the styles were wet and sticky, probably from the secretion of the glandlike cells in the conducting cavity. Out of one hundred flowers pollinated, forty-eight fruits

were set. This percentage of set was about the same as the average for properly cross-pollinated flowers of the variety that season. While no histological investigation of the material was made, it is evident that styles are neither necessary for the germination and development of pollen tubes nor for fertilization.

It was interesting to observe the behavior of pollen tubes, which pushed into the tissue of the styles and hence exhibited an abnormal conduct. From the stigma, or sometimes from the side

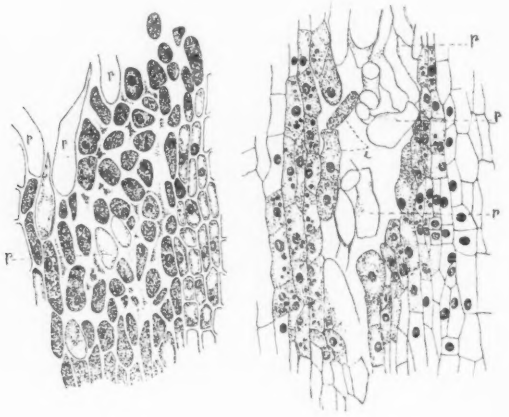

FIG. 8 FIG. 9

FIGS. 8, 9.—Fig. 8, longitudinal section through part of style, showing penetrating pollen tubes and somewhat macerated tissue; fig. 9, later stage of fig. 8; *i*, isolated cell; *p*, pollen tube.

of the style, the tubes grew into the tissue singly or in small bundles. In the first step of this penetration, it was not evident whether the cuticular layer was dissolved or whether some wounded part was attacked. The cells of the tissue penetrated by the tubes were rich with granular cytoplasm, and showed an apparent thickening of the membrane. This thickening of the cell wall was not due to the growth of the membrane itself, but was a result of dissolution, which occurred especially in the middle and secondary lamellae. The dissolution gradually proceeded, and finally some cells became isolated (figs. 8, 9). In general the cells increased

in size during the process of isolation. Isolated cells had thin walls. This maceration of tissue was not ascribable solely to the action of growing tubes. As reported by PATON (7), the pollen tube has the ability to dissolve the membrane material through the action of some enzyme contained in its secretion, but some part of this reaction seemed to be participated in by the tissue itself. The changes in the cell content and the growth of the cell in size indicated this. As this process of isolation was very slow, however, the growth of the tubes was markedly inhibited. Occasionally their tips became swollen, as shown in fig. 9, or sometimes the direction of growth turned toward the other side. From his study of the behavior just described, the writer suspects that OSTERWALDER'S (4) report of the development of the pollen tubes upon selfed flowers, and his assertion that the tubes grew very slowly or became so thick that they could not pass through, were based on observations of such abnormal passage.

Further questions on the problem of self-sterility in apples are these: (1) After the tubes reach the ovary core, does the ovule itself inhibit the penetration of the tubes into it, or do some other factors exist which obstruct the fertilization? (2) Even though fertilization might occur, is it not possible that the subsequent development of the embryo would lack vigor? These phases are not within the scope of this experiment, and further investigations are desirable.

The writer wishes to express his thanks to Professor E. L. OVERHOLSER, who suggested the problem, gave valuable advice, and helped in other ways.

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BRIEFER ARTICLES

REDUCTION OF MICROSCOPE FATIGUE

(WITH THREE FIGURES)

In the course of biological research or routine examinations which involve long hours at the microscope, the efficiency and output of the worker in many cases are limited by the effects of microscope fatigue, which manifests itself not only in the form of eyestrain and headache, but also in general lassitude and depression. Although it appears impossible to eliminate microscope fatigue entirely, much can be done, with a modicum of experiment, to reduce it to reasonable limits. The fatigue may usually be traced to two general causes, namely, faulty working position and incorrect illumination.

Faulty working position can usually be changed by intelligent trial of various positions of the head and body in relation to the microscope and working table, until a comfortable posture is found in which no muscle is unduly strained.

Incorrect illumination is probably an even more common cause of fatigue. Daylight is still used in many laboratories for the majority of microscopic work on grounds of simplicity; but for all work of a critical nature, where it is required to obtain the best results possible with available equipment, artificial light is the rule in modern practice, since it is easier to obtain a critical image, and the light does not vary in intensity from hour to hour. In either case it is seldom that the light used is so adjusted as to be entirely comfortable to the eye, and, even if it is, the changing of an objective or an eyepiece, or any alteration of the substage adjustments, markedly varies the intensity of the illumination. Under such circumstances the worker is tempted to alter his substage until the light is more comfortable, usually by closing the iris diaphragm, thus reducing the numerical aperture of the condenser, to the detriment of the image.

To overcome this difficulty an equipment was designed in which the intensity of illumination could be varied without in any way affecting the adjustment of the optical system, and a method of light control for microscopes was proposed,[1] using a modification of the "Pointolite." This lamp, which is manufactured by an English firm, although a similar

[1] DENHAM, H. J., Microscope illumination and fatigue. Nature **108**:369,496. 1921.

form has recently been put on the market in America, is an ingenious modification of the well known thermionic valve, and consists in its simplest form of a glass bulb filled with a gas at reduced pressure, with a spiral filament of tungsten, which can be raised to incandescence, and a tungsten bead which forms the positive pole of an arc circuit. The arc is struck by raising the ionizing spiral to a white heat, when ionization by collision takes place, and the gas becomes sufficiently conducting to allow current to flow across the gap, forming an arc which persists when the current through the spiral is broken. The source of light is the tungsten bead, which is small enough to form an almost ideally small point source of light, and to satisfy the optical conditions demanded by microscope illumination. The lamp is made in several sizes, from 30 to 5000 cp. In laboratories, 100 cp. is the type usually employed for microscope work, but it has been found that the 30 cp., when used with a properly designed parallelizing lens, gives more than adequate illumination for the highest powers used in visual work, and quite enough for microphotography, exposures of three seconds being sufficient at a magnification of 700 with "ordinary" plates and a Wratten 45 filter. Control of the intensity of the light can be effected by inserting a sliding resistance into the arc circuit, enabling the temperature of the tungsten bead to be reduced from incandescence to a dull red glow, or, when a monochromatic filter is used, which is advisable, from "full" radiation almost to "zero." The control resistance is fastened to the bench, where it can be found and adjusted without moving the eye from the eyepiece, and when any change is made of either eyepiece, objective, or substage adjustment, the hand almost subconsciously makes the change necessary in the intensity of the illumination.

Apart from the reduction of fatigue, the system appears to have the advantage of rendering fine detail more easily visible, and in cytological preparations either the chromatin or the achromatic structures can be emphasized at will. When used with the Abbé drawing apparatus, the control obviates the usual difficulty of balancing the illumination of the field and the drawing point.

The special circuit required for the Pointolite is shown in fig. 1. The starting resistance *A* and the arc resistance *B* are supplied by the makers in one unit, but can easily be built up as required. The values of these and the variable resistance have already been published elsewhere.[2] The latter may be of any convenient pattern, but should be so shielded as to avoid the risk of accidental shock. Special patterns of Pointolite are made for use with storage batteries and for alternating current.

[2] DENHAM, H. J., The projection microscope. New Phytol. **21**:163. 1922.

Since the first publication of the system, BARNARD[3] has drawn attention to two other methods of obtaining variable illumination without lowering the temperature of the light source. The first employs two neutral glass wedges, which are moved across each other and in front of the illuminant by a rackwork mechanism. If the wedges were optically perfect, and the mechanism could be controlled from the front of the bench, without the removal of the eye from the microscope, this device

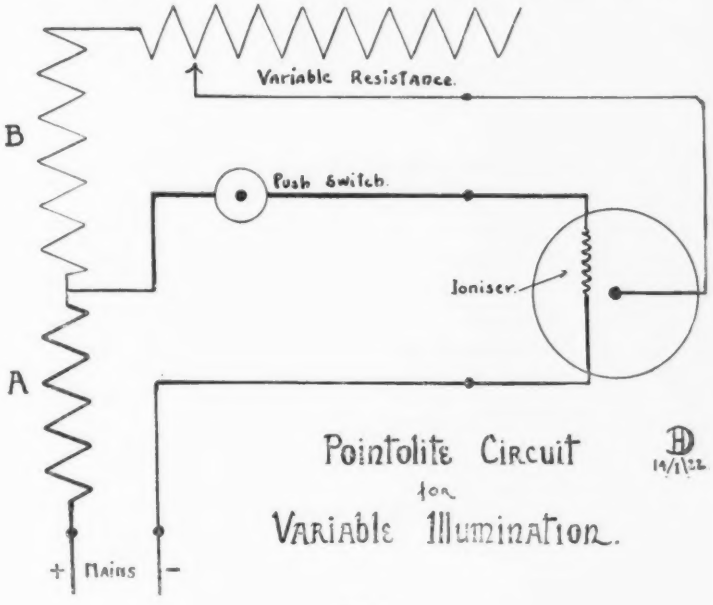

FIG. 1

would be of the greatest value where electric lighting is not possible. The second method, used by BARNARD, involves the construction of a number of platinized glass screens, of definite light-passing properties, calculated for the theoretical light transmission of every combination of objective, eyepiece, condenser, and color filter used. This would appear rather a complicated undertaking for most laboratories, although the new Wratten neutral screens might be pressed into service. The method of mounting the large number of screens necessary is not specified.

[3] BARNARD, J. E., Microscope illumination and fatigue. Nature **108**:468, 566. 1921.

The use of color filters to give a more or less monochromatic light has been recommended strongly by SPITTA[4] and other investigators, and is now normal practice in critical work. The color of the screen used is governed by the staining of the material under examination, and by the degree of resolution required of the objectives employed; the shorter the dominant wave length of the light passed, the greater the resolution. Many workers find blue light extremely tiring, however, and for routine work prefer green as more restful. In cytological examinations green light reinforces Haidenhain staining to a remarkable degree.

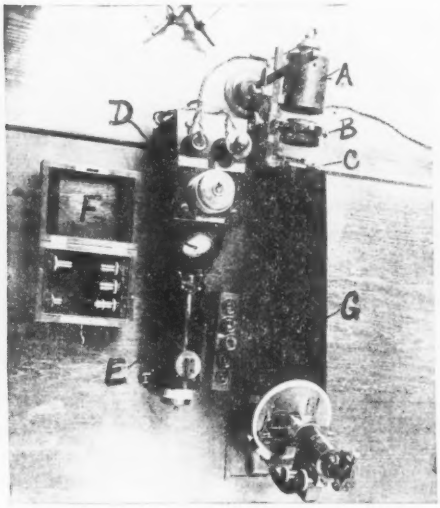

FIG. 2

The last factor of microscope fatigue from the visual side depends on the relative brilliance of the field of the microscope and the general illumination of the laboratory. This again seems a matter of individual preference, and it has even been recommended that microscopy should be done in a darkened room when artificial light is used. This appears to be quite as unnecessary as it is depressing, but some arrangement should be made by which the general illumination of the working bench could be regulated. When the Abbé drawing apparatus is much used, the provision of a small reading lamp to illuminate the drawing surface is a further convenience.

In the accompanying illustrations fig 2. shows a typical arrangement of a microscope table as seen from above. *A* is the Pointolite in its adjustable housing with the parallelizing lens (*B*) and the screen holder (*C*). *D* is the starting resistance, and *E* the extra, adjustable resistance, while *F* is a small ammeter to show the current taken. *G* is a screen of black fabric which covers the space between the Pointolite and the substage mirror, and prevents stray daylight from being reflected up through

[4] SPITTA, E. J., Microscopy. ed. 3. London. 1920 (p. 493).

the condenser as a colored haze round the image. The Pointolite and its resistances are screwed to the table top, and the microscope drops into wooden stops which hold it in place. Fig. 3 shows the table as arranged

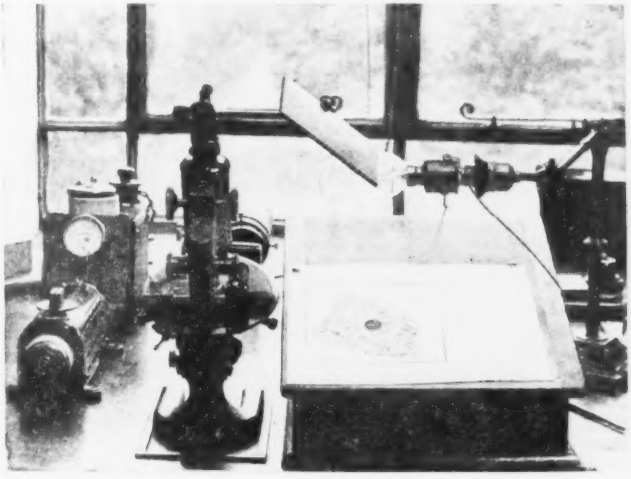

FIG. 3

for use with the Abbé drawing apparatus, with the shade removed from the reading lamp used to illuminate the drawing surface and showing the Dimmolite adaptor.

Summary

Microscope fatigue in the majority of cases is due to faulty working position or incorrect illumination. For correcting the latter a system of variable artificial light is recommended, together with a suitable screen or light filter. A suitable source of light is the Pointolite, which is briefly described, with an extra, variable resistance in circuit, and so arranged that it can be altered without removing the eye from the eyepiece. Two other methods of obtaining variable illumination by the use of neutral screens have been suggested. It is claimed that the use of the Abbé drawing apparatus is considerably simplified by the employment of variable illumination, particularly if the lighting of the drawing surface is also artificial.—HUMPHREY JOHN DENHAM, *British Cotton Industry Research Association, Shirley Institute, Didsbury, England.*

CURRENT LITERATURE

BOOK REVIEWS

Physiology of ascent of sap

The latest volume by BOSE[1] deals with the problem of the ascent of sap in plants. His methods of investigation and presentation follow lines already familiar to his readers, such as electric detection of physiological changes, and automatic recording devices of considerable ingenuity, some of which receive high-sounding names. In general, he believes that vital activity is solely responsible for sap ascent. He claims that a layer of cells in the cortex, lying next to the endodermis, pumps the sap upward by pulsating activity of the individual cells. Each successive cell up the stem in this active layer is in a slightly different phase of expansion or contraction, and the distance from any given cell up to the next cell that has the same phase of activity, is called the hydraulic wave length. This wave length differs for different plants: *Chrysanthemum*, 100 mm.; *Musa*, 50 mm.; *Canna*, 40 mm. Anything that changes the wave length or wave frequency changes the rate of sap flow. Thus increased temperature lengthens the hydraulic wave and hastens sap rise, etc.

One feels as he reads the early chapters that the present state of our knowledge and beliefs regarding sap ascent has been grossly misrepresented. The really important contributions to the cohesion theory have nearly all appeared since EWART'S translation of PFEFFER'S work, yet BOSE summarizes the historical background by citations of literature, none of which is less than twenty years old. The only reference to DIXON'S work is to the original paper in 1895, and RENNER'S contributions are not even mentioned. The time has passed when anyone can summarize our present knowledge of any great physiological processes by quotations from PFEFFER.

Not only is the status of the subject misrepresented, but the data furnished by the author are so grossly misinterpreted at places that one loses confidence in the argument as a whole. For instance, the rate of sap ascent is calculated from the time required for a wilted leaf to begin to regain turgidity when water is applied to the cut end of the stem. He considers that the water taken in at the cut end has traveled the entire distance to the leaf in the time noted. It would be as logical to assume that the water we draw from the tap has traveled from the pump to the tap while we are drawing it. Again, he claims that transpiration, in the sense of active secretion of liquid water by the leaf cells, occurs in the absence of evaporation. This may be true in the case of certain leaves that normally guttate, but certainly cannot be true of transpiration in

[1] BOSE, J. C., Physiology of the ascent of sap. 8vo. pp. xv+277. New York, London: Longmans, Green and Co. 1923.

general in the plant kindgom. Students can easily procure positive evidence that even very active types of leaves, if relieved of all saturation deficit before testing, do not show water excretion, nor transpiration, as BOSE claims, when evaporation is precluded.

These are minor points, but the only facts that one can accept as proved are the facts of peculiar electrical responses on the part of certain living cells. The rest is largely assumption. To the reviewer there seems to be nothing in BOSE's work to prove that the inner cortical cells carry any sap by pulsation. All the facts that we do know regarding sap ascent indicate that the xylem carries the water, and that evaporational disturbance of water equilibrium in the leaf is the potent physical force which leads to sap ascent.—C. A. SHULL.

Botanical pen-portraits

Under this title, MOLL and JANSSONIUS[2] have developed an elaborate system of plant description, based upon the Linnean system for taxonomy. It is claimed that this method "leads to completeness, avoids unnecessary repetitions, gives a maximum of information by a minimum of words and in a form capable of being consulted in whole or in part." It is really a presentation of the art of description, using as illustrations 100 drug plants, whose microscopic characters are described in detail in taxonomic style, including all parts of plants used in pharmacy. Several of the descriptions contain the macroscopic characters also. In looking through the descriptions, it is very evident that the result is compactness of statement, and an organization that permits easy consultation in reference to any detail. In short, it is an extension of the taxonomic method of description to include all structures. It would change the investigation of literature from the laborious reading of papers to the brief consultation of a dictionary. It is a bold attempt, but perhaps no more so than the original establishment of a uniform taxonomic method of description.—J. M. C.

Poison ivy

MCNAIR[3] has published a volume on poison ivy with which botanists should be familiar. The investigation recorded involves pharmacology, botany, and chemistry. The morphological study has given the method of formation, location, and means of transmission of the poison, while the pathological results show its manner of action on the body. As a result of these co-operating methods of attack, combined with a chemical knowledge of the structure of the poison, what is called "a rational remedy" has been secured. Since this is probably the most common cutaneous eruption caused accidentally by plant substances, a sure remedy is most desirable.—J. M. C.

[2] MOLL, J. W., and JANSSONIUS, H. H., Botanical pen-portraits. pp. viii+472. *figs. 111.* The Hague: Martinus Nijhoff. 1923. 30 guilders.

[3] MCNAIR, J. B., *Rhus dermatitis* (poison ivy), its pathology and chemotherapy. 8vo. pp. xi+298. *pls. 3. figs. 15.* Univ. Chicago Press. 1923. $4.00.

MINOR NOTICES

British fungi.—RAMSBOTTOM[4] has published a very convenient handbook for the recognition of the "larger British fungi," based on the "guide to Sowerby's models of British fungi," which are on exhibition in the British Museum. The present work extends the range of fungi to include all the British genera of Basidiomycetes. The descriptions have also been revised and enlarged, and "additional matter of economic and biological interest has been included." This should prove a very useful book to those interested in the more obvious British fungus flora.—J. M. C.

NOTES FOR STUDENTS

Helminthosporium foot rot of wheat.—STEVENS,[5] in his investigation of *Helminthosporium*, has developed both the morphology and genetics of the situation. These two phases are presented separately in the following reviews.

In this paper STEVENS presents the results of an extensive study of many strains of *Helminthosporium sativum* P.K.B., which he considers the cause of a foot rot of wheat in Madison County, Illinois. The disease was first observed in 1919. It was first thought by federal pathologists to be identical with the take-all disease caused by *Ophiobolus cariceti* (Berk. and Br.) Sacc. (*O. graminis Sacc.*), but *Ophiobolus* could not be found in the diseased tissues. The name, "so-called take-all," of unknown causation, then was applied. In December, 1919, however, STEVENS presented a paper at the meeting of the American Phytopathological Society, in which he cited evidence that *Helminthosporium* sp. was the cause of the disease. In his latest publication STEVENS maintains that the disease is caused by *H. sativum*, which consists of many slightly but consistently different races, elementary species, or biologic forms. Evidence of the causal relationship of one of these strains, strain no. 1, is given in detail, and corroborative work was done with several other strains.

Helminthosporium was the only organism consistently present in the lesions of wheat plants affected with foot rot. The fungus was isolated and studied carefully in pure culture. The cut ends of wheat plants were inoculated by placing them in contact with colonies of the fungus; seedlings were inoculated under aseptic conditions in Petri dishes; young wheat shoots were inoculated in rag-doll germinators, and roots were inoculated in rag-dolls and in soil. Infection resulted from all methods of inoculation, and the organism was recovered easily.

The germ tubes of *Helminthosporium*, according to the author, may penetrate directly through the epidermis of wheat plants, but more frequently they

[4] RAMSBOTTOM, J., A handbook of the larger British fungi. pp. 222. *figs. 141*. London: British Museum. 1923. *7s. 6d.*

[5] STEVENS, F. L., The *Helminthosporium* foot rot of wheat, with observations on the morphology of *Helminthosporium* and on the occurrence of saltation in the genus. Bull. Ill. Nat. Hist. Survey 14. pp. 76–185. *pls. 7–34. figs. 23.* 1922.

enter by pushing between the cells. Numerous appresoria are formed; from these the "penetrating mycelium" is produced. This pierces the cell wall, changing its staining reactions, and then grows inside the cells. On the inner wall of penetrated cells a "callus" is formed. This seems to be "of the nature of a precipitation" caused by the toxic action of the pathogene. The mycelium penetrates cell after cell, causing at last slight callus formation in each living cell. Many such calluses may be formed, even at some distance from the point of penetration. The mycelium may grow so luxuriantly within the cell as to form a pseudoparenchyma-like mass. Inoculations were made with *Alternaria*, *Sterigmatocystis*, *Penicillium*, and several other supposed saprophytes, but none caused infection, although *Alternaria* grew somewhat in the cortex of roots.

Some work was done to ascertain the host range, and it was found that corn, barley, rye, sorghum (*Holcus* sp.), Sudan grass (*Holcus sorghum sudanensis*), common millet (*Chaetochloa italica*), German millet (*Chaetochloa italica germanica*), and Amber cane (*Holcus sorghum*) were susceptible. Beans and red top (*Agrostis palustris*) did not become infected. Leaf spots also were produced on barley and wheat; rye leaves were killed; but oats were somewhat resistant.—E. C. STAKMAN.

A collection of some 46 races, isolated by STEVENS from infected wheat or other grains or sent him by correspondents, indicated that distinct forms of this fungus exist in nature. They differed often markedly in gross appearance and in microscopical details, and as well in response to changes in environmental conditions. A biometrical study of size, shape, and septation of spores showed the distinctness of closely related forms, but since in many cases the differences were slight and brought out only by a comparison of their respective graphs, it seemed best to consider these different races elementary species, and to label them with numbers rather than to attempt to dignify the differences by specific names. Certain groups were recognizable, however, which seemed worthy of specific names. Of these the group species *Helminthosporium sativum* contained the elementary species responsible for the foot rot disease. The race H1 of this species was studied intensively in regard to its variability.

The morphological characters were more or less modifiable by changes in quantity and quality of nutrient, in humidity, and in temperature. The chief interest in the paper, however, centers upon the sudden appearance of variants which often occur when the fungus is grown upon a standard medium and under constant conditions. These variants are called "saltations." Single spores sown in Petri dishes give rise to mycelial colonies in which the saltations may show toward the periphery of the growth as sectors differing more or less distinctly in appearance from the normal mycelium. Sowings of single spores frequently gave rise to saltants without the preliminary growth of a normal mycelium. Saltation is evidenced in general colony character; rate of growth; spore production; spore clusters; spore length, breadth, septation, and shape; mycelial characters, color, zonation, and sclerotial formation. Certain saltants

differed so markedly from their parent as to far exceed the usually accepted specific limits. Generally the variants were less vigorous in growth, although in certain types the reverse was the case. In the main the saltants were permanent in character whether propagated by mycelial transfers or by spores, although what appeared to be reversions sometimes occurred. After a long series of transfers, certain races were found to be distinctly different from the type first isolated, presumably through saltation to a more vigorously growing form. It is not unreasonable to conclude, therefore, that saltation has been a method of origin of the diversity in *Helminthosporium* found in nature. Apparently races differed in their tendency toward saltations, race H_1 of those investigated being most inclined to give rise to new forms. Since most of the variants were obtained from abnormal sectors of mycelial colonies (126 such sectors were studied), it is somewhat difficult to calculate the frequency of saltation. It is estimated, however, to be much more frequent than the bud sports in potatoes, with which the phenomenon of variation in *Helminthosporium* is compared.

STEVENS prefers the word saltation to the more usual term mutation on account of the differences in the use of the latter word, on account of the limited knowledge of the cytological conditions in the genus, and because of the uncertainty as to whether the species investigated has sexual stages. He believes that segregation from a heterozygous condition in his mycelia, nuclear transference during anastomosis, or cytoplasmic contamination by such anastomosis are possible causes of the origin of his variants which would preclude the use of the term mutation. Mutation, however, is a mere descriptive term which lightly covers our ignorance in regard to the processes involved in the origin of the variants in question. We do not know what a gene is nor what processes bring about its change in gene mutations. It seems of questionable service, therefore, to attempt to introduce a new term to emphasize our ignorance as to how heritable variations have arisen. Chromosome mutation is not suggested as a possible cause of the somatic variations in *Helminthosporium*, and yet changes in the chromosome number are known to be the cause of somatic mutations in certain cases, and may prove to be a common cause of bud sports when they come to be more thoroughly investigated. Segregation, from a heterozygous mycelium, is an unlikely explanation; first, because the fungus, if devoid of a sexual stage, is likely haploid and a strictly $1n$ plant cannot be heterozygous; second, even if the fungus were heterozygous there is no satisfactory evidence that so-called somatic segregation is not rather somatic mutation. The natural reason why somatic mutations when recessive are more common from a heterozygous plant is because if the plant were a homozygous dominant, both the chromosomes would have to mutate in the same locus in order to show somatically, while if the plant were a heterozygous dominant, a single gene mutation could be evident at once. The reviewer has succeeded in finding a race of *Portulaca* in which the heterozygous was distinct from the homozygous condition, and discovered that somatic variants occurred

both to homozygous dominants and to homozygous recessives, judging from the color changes in the petals. He believes, moreover, that there is no good evidence that gene mutations occur more frequently during reduction division than during any other stage, although obviously they would be more likely to survive and show in the offspring if they took place in the formation of the gametes or at an early stage in the development of the zygote. "Nuclear transference," if it involved an increase in the number of chromosomes, would be comparable with the chromosomal mutations in *Oenothera* and *Datura*, while "cytoplasmic contamination" would be a rather novel method of bringing about permanent genetic change.

STEVENS summarizes his conclusions as follows. "The cases reported by BRIERLY, BURGEFF, BLAKESLEE, and CRABILL, and my own work reported herein, all based on single-spore culture and carried under observation for sufficient time to give assurance of permanence, constitute complete proof of the occurrence of the phenomenon of sudden change in character among the fungi. The evidence from EDGERTON, SHEAR and WOOD, and DASTUR, while not so complete, is strong collaterally. It would seem from all this evidence that this phenomenon is common and widely distributed among the fungi, although unquestionably it is more common in some species and races than in others."

Geneticists should be grateful to STEVENS for his intensive study of mutability in one species of the fungi, a group in which too little genetic work has so far been attempted.—A. F. BLAKESLEE.

Solution of Oenothera problem.—For a decade or more it has been recognized by geneticists that *Oenothera* does not clearly conform to the Mendelian principles of heredity that have been so well established for other organisms. The prominence given to *Oenothera* by DEVRIES' classic experiments early stimulated a wholesale investigation of inheritance in this form, but the seemingly chaotic results obtained and the numerous imaginative explanations proposed have caused most geneticists to conclude that *Oenothera* is "a law unto itself." SHULL[6] has recently straightened out part of this chaos by an interpretation which conforms perfectly with Mendelian principles. He points out that the peculiarities of the *Oenothera* case arise from two unusual situations: (1) from the first, systems of lethal factors have existed which have consistently obscured breeding results by eliminating certain of the expected progeny classes; (2) with one exception, all the hereditary characters that have been investigated are linked together on the same chromosome pair that carries these lethals.

The lethals are of two sorts, zygote lethals and gamete lethals. Zygote lethals cause the death of zygotes which lack the factor in question. Thus self-fertilization of the heterozygote *Ll* results in a progeny of which 25 per

[6] SHULL, G. H., Linkage with lethal factors the solution of the *Oenothera* problem. Eugenics, Genetics, and the Family 1:80–99. 1923.

cent are *ll*, and these die. (*L* may be regarded as a vital factor, its absence bringing the lethal effect.) This system brings confusing results when one is considering other factors that are linked on the same chromosome with the lethal, for certain expected classes are eliminated with the *ll* zygotes. The situation is further complicated by the existence of balanced lethals (previously described by MULLER[7]). *O. Lamarckiana* has two sets of lethals on the same pair of chromosomes. Each of these lethals exists in the heterozygous condition, with the dominant members on the opposite chromosomes of the pair. Thus one chromosome carries L_1 and l_2, while its mate carries l_1 and L_2. As a result, inbreeding *O. Lamarckiana*, which produces the following ratio among zygotes: 1 L_1–l_2, L_1–l_2 : 2 L_1–l_2, l_1–L_2 : 1 l_1–L_2, l_1–L_2 . . . ., actually develops only those individuals of the middle type, the other zygote types being eliminated by one or the other of the lethals. This conforms with the observed fact that one-half of the seeds fail to germinate. It is evident that *O. Lamarckiana* must continue to breed true to this heterozygous condition. This does not necessarily imply that *O. Lamarckiana* is of hybrid origin, since the existing condition might have been brought about by the appropriate sequence of factor mutations in what was originally a pure race. Since *O. Lamarckiana* breeds true in this way to a heterozygous condition for both of the lethal factors, this same mechanism causes it to breed essentially true to a heterozygous condition with respect to other factors that are closely linked on the same chromosomes with the lethals. If the factor pair *Aa* is closely linked with the lethals, inbreeding will perpetuate the *Aa* condition, since *AA* will be eliminated with the *A*–l_1–L_2, *A*–l_1–L_2 zygote, and *aa* will be eliminated with the *a*–L_1–l_2, *a*–L_1–l_2 zygote. Occasional crossing over between the *A* locus and the lethals will permit the appearance of *aa* in a very few individuals of the constitution *a*–L_1–l_2, *a*–l_1–L_2. Since this *aa* is a new phenotype, appearing in very small numbers among the "normal" progeny and subsequently breeding true, it is not surprising that DEVRIES originally regarded such forms as "mutants."

When one realizes that all but one of the investigated characters of *O. Lamarckiana* are linked with these lethals, and when one further realizes that some of the other species of *Oenothera* also possess various combinations of these same lethals, one can appreciate readily the complexity of the breeding results that have been obtained.

Other unusual situations are introduced by the gamete lethals, which destroy, or at least render functionless, certain classes of gametes. In *O. biennis* and *O. suaveolens* the wild types carry a recessive factor *S* for sulphur colored flowers, closely linked with a sperm lethal *ls*, while the mate to this chromosome carries the corresponding dominant factor *S* for yellow flower color, linked with an egg lethal *le*. When such a plant produces eggs, the 50 per cent which receive the yellow factor *S* are prevented from functioning by the egg lethal with which *S* is linked, and the sperms which carry the sulphur

[7] BOT. GAZ. 72:182. 1921.

factor *s* are prevented from functioning by the sperm lethal linked with it. Thus it is that the functional eggs carry only *s*, while the functional sperms carry only *S*. In crosses between such a yellow race and a sulphur race offspring resemble the pollen parent whichever way the cross is made.

By means of the linkage relations and these two types of lethals, SHULL clarifies many of the *Oenothera* perplexities. Whether all of the *Oenothera* perplexities will be susceptible to a similar interpretation remains to be seen.—M. C. COULTER.

Somatic chromosome cycle.—The well known difficulties attendant upon observation and interpretation of nuclear phenomena no doubt are responsible for the differences of opinion that have existed among competent investigators of this subject. Recently, however, one view appeared to have swept the field, and cytologists were coming to the opinion that the nucleus consisted of but one constant element, chromatin, arranged into constant separate bodies, the chromosomes, and an inconstant body, the nucleolus, disappearing at mitoses, and composed of reserve material of some sort. The periodic changes in the appearance of the chromosomes were held to be due to vacuoles, the longitudinal split occurring in the prophase, when the chromosomes had become slender single threads, through breaking down of vacuoles and straightening of the remaining network.

A new view is now presented by MARTENS.[8] In the chromosome of *Paris quadrifolia* he sees two constituents, one "chromonématique," which may be translated as "chromonema," a zig-zag thread which may be distinguished at all times, and the other an achromatic ground substance surrounding the chromonema and impregnated with nucleolar matter during the metaphase and anaphase, thus rendering the chromonema difficult to detect at these stages.

The appearance of vacuolation is really due, he claims, to this zig-zag thread, which elongates and becomes thin during the prophase, but which does not split at this stage. Instead it becomes shorter and thicker, and in the late prophase the cross threads thin out and separate, leaving innumerable chromatin granules in two rows along opposite sides of the chromosome, which is ribbon-like. These granules coalesce and reform zig-zag threads at about the time the homogeneous achromatic substance splits. The split halves remain associated for some time, the chromonema being visible in cross-section even in the metaphase, as horseshoe-shaped with hollow surfaces opposed, and filled with the ground substance now impregnated with nucleolar matter. In the telophase the nucleolus reappears as the achromatic substance becomes fainter. Meanwhile the chromonema takes on a dual appearance, but does not really split. Loose ends of chromosomes may be distinguished in the resting stage, indicating the maintenance of their individuality.

[8] MARTENS, PIERRE, Le cycle du chromosome somatique dans les Phanérogames. I. *Paris quadrifolia* L. La Cellule **32**²:331–428. *pl. 4.*

The difficulties of technique are such that it is impossible to express an opinion on this work. From the standpoint of the chromosome theory of heredity, the thesis that the only non-homogeneous substance in the nucleus divides transversely at an infinite number of points, and that alternative portions of this thread should coalesce to form the threads of the daughter chromosomes is not attractive. Indeed, it would appear impossible to harmonize the two in a practical way. Of course one should not allow a priori considerations to sway one's judgment and interpretation. In this case it is obvious that the author has not attempted to square his views with current theories.

It is a pity that this important question has not appeared susceptible to attack from some other angle. If the great difficulties attendant upon the use of ultra-violet rays could be overcome, it might be possible to secure microphotographs of successive stages of mitosis of the same cell under comparatively normal living conditions. This may be accomplished some day, and, as chromatin is opaque to ultra-violet rays, it might throw light on the problem. Meanwhile it is to be hoped that the author will turn his attention to the reduction division. The promised results of his work on other plants will be welcomed.—R. O. EARL.

Taxonomic notes.—GLEASON[9] has described a new genus (*Windsorina*) of Rapataceae, a small family of South American plants. He discovered the plant in a dense tropical forest of British Guiana.

SARGENT[10] has added eight new species of *Crataegus* to the long list already described under this polymorphic genus.

MURBECK[11] has published an account of the flora of Morocco, illustrated with excellent plates. Numerous new species are described and also a new genus (*Pantorrhynchus*) of Cruciferae.

MARKGRAF[12] has described a new genus (*Ephippiocarpa*) of Apocynaceae from South Africa, related to *Tabernaemontana*.

GAMBLE[13] has described a new genus of bamboo (*Neohouzeaua*) from Burma.

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[9] GLEASON, H. A., *Windsorina*, a new genus of Rapataceae. Bull. Torr. Bot. Club **50**:147–152. *pl. 7.* 1923.

[10] SARGENT, C. S., Notes on North American trees. XI. New species of *Crataegus*. Jour. Arnold Arboretum **4**:99–107. 1923.

[11] MURBECK, Sv., Contributions à la connaissance de la flore du Maroc. I. Ptéridophytes-Legumineuses. pp. 76. *pls. 12. figs. 4.* II. Géraniacées-Composées. pp. 68. *pls. 7. figs. 8.* Lund. 1922.

[12] MARKGRAF, FR., *Ephippiocarpa*, eine neue Gattung der Apocynaceen aus Südostafrika. Notizbl. Bot. Gart. und Mus. Berlin-Dahlem **8**:301–311. 1923.

[13] GAMBLE, J. S., *Neohouzeaua*, a new genus of the bamboos. Kew Bull. 1923. no. 2. pp. 89–93.

WATSON[14] has published a revision of *Heliocarpus* (Tiliaceae), a genus of the American tropics. He recognizes twenty-two species, three of which are described as new.

KAUFFMAN[15] has given the result of two trips to the higher Rockies of Colorado to investigate the mycological flora. The list of species is surprisingly long, and includes descriptions of one new species of Ascomycetes and ten new species of Basidiomycetes, *Cortinarius* including five new species.

LAUTERBACH[16] has described 122 new species of orchids from Africa, representing twenty-five genera, a good illustration of the wealth of the African flora that remains to be explored.

MILDBRAED[17] has described a new genus (*Zygotritonia*) of Iridaceae from Africa, including three species, two of them new, and one transferred from *Tritonia*.

SCHLECHTER[18] has described a new genus (*Euthamnus*) of Gesneriaceae from Africa. In the same contribution he also describes twenty-seven new species, representing six genera.

SHAW[19] has recently published four papers dealing with the Volvocaceae in the region of Manila. The descriptions are very detailed and well illustrated. Among the forms presented, in addition to new species and new combinations, the following new genera are described: *Janetosphaera*, *Merrillosphaera*, and *Copelandosphaera*.—J. M. C.

Crown gall and cancer.—The comparison of crown gall with cancer and tumors has been based chiefly upon the experimental production of secondary tumors and vegetable "teratomas," or crown galls, bearing leafy shoots which have no relation to preformed buds; upon the formation of secondary tumors following the intrusion of "tumor-strands" into healthy tissue; and upon the reproduction in the secondary tumors of the structure of the tissues in which the primary tumors have developed. Recently ROBINSON and WALKDEN[20]

[14] WATSON, E. E., The genus *Heliocarpus*. Bull. Torr. Bot. Club **50**:109–128. 1923.

[15] KAUFFMAN, C. H., The mycological flora of the higher Rockies of Colorado. Mich. Acad. Sci. **1**:101–150. *pls. 30–34*. 1921.

[16] LAUTERBACH, C., Neue Orchidaceen Papuasiens. Bot. Jahrb. **85**:50–154. 1922 and 1923.

[17] MILDBRAED, J., Iridaceae Africanae. Bot. Jahrb. **85**:230–233. 1923.

[18] SCHLECHTER, R., Gesneriaceae Papuanae. Bot. Jahrb. **85**:255–304. 1923.

[19] SHAW, W. R., *Janetosphaera*, a new genus and two new species of *Volvox*. Philippine Jour. Sci. **20**:477–508. *pls. 5*. 1922.

———, *Merrillosphaera*, a new genus of the Volvocaceae. *Idem* **21**:87–129. *pls. 8*. 1922.

———, *Copelandosphaera*, a new genus of the Volvocaceae. *Idem* **21**:207–232. *pls. 4*. 1922.

———, *Merrillosphaera africana* at Manila. *Idem* **22**:185–218. *pls. 7*. 1923.

[20] ROBINSON, WILFRED, and WALKDEN, H., A critical study of crown gall. Ann. Botany **37**:299–324. *pls. 2*. 1923.

have completed a pathological and histological study of crown gall in *Chrysanthemum frutescens* and *Nicotiana affinis*, from which they conclude that crown gall has only a general similarity to animal tumors.

The "teratomas" in the leaf of the *Chrysanthemum frutescens* consist of leaf tissue developed on the adaxial side of the vascular bundles, as a result of the stimulation of bacteria in the protoxylem, so that the growth is comparable with that arising from adventitious buds on certain leaves. In other words, the growth is not a "pseudo-stem" in a leaf caused by the intrusion of a tumor-strand, but a leaf hypertrophy due to the direct stimulation of bacteria. True tumor-strands were not observed, but "bacteria advancing in the intercellular spaces and the protoxylem fully explain the development of the tumor-tissue in the neighborhood of their path, and also the existence of strands of such tissue connecting secondary with primary galls. There is therefore no necessity to assume the stimulation of cells at any considerable distance from the active bacteria."

Galls arising on *Chrysanthemum frutescens* as a result of inoculation with *Bacterium tumefaciens* differ in regard to the appearance of their surfaces. Primary galls following the inoculation of cut surfaces of shoots are rough externally, and resemble natural galls, while the secondary galls and tumor-strands are smooth. The rough galls have bacteria on the surface, but the smooth ones are infected in the intercellular spaces and vessels. No bacteria were found in living cells. The distribution of the bacteria explains the difficulty heretofore experienced by various workers in locating bacteria in surface-sterilized material, and it also "throws light on the form of these galls. . . . . The organisms present over the hemispherical outer surface of the gall provide the continued stimulus which accounts for the fairly uniform meristematic activity in a region of the gall comparatively close to the surface." Just how this stimulus is applied by organisms on a corky surface in such a way that it reaches the dividing cells, is not discussed.

From an economic standpoint, the distribution of *Bacterium tumefaciens* on the surface of crown galls is very important if it holds true generally for natural galls. It may explain the extensive attacks of crown gall in orchards under irrigation, where the reviewer has seen plantings of more than ten acres almost uniformly infected and dying.—J. G. BROWN.

Apogamy in Calycanthaceae.—According to JÖNSSON,[21] in the Calycanthaceae hypodermal cells of the nucellus form a mass of sporogenous tissue from which arise megaspore mother cells, some of which degenerate by undergoing vegetative divisions. Other mother cells of the central mass divide, and some of the daughter cells develop into embryo sacs. LONGO[22] also states

[21] JÖNSSON, B., Om embryosäckens utveckling hos Angiosperma. Lunds Univ. Arsskrift **16**:1881.

[22] LONGO, B., Un nuovo carattera di affinità tra le Calycanthaceae e le Rosaceae desunto dall' embriologia. Rend. R. Accad. Lincei **7**:1898.

that there is a mass of sporogenous cells in *Calycanthus*, and PETERS[23] holds that the nuclei of the embryo sac mother cells undergo a reduction division, so that each nucleus of the embryo sac contains the haploid number of chromosomes. PETERS holds that some of the nuclei of the embryo sac degenerate, while others go to form the endosperm, so that the endosperm contains haploid nuclei. He states also that the embryos are developed apogamously from nucellar cells which grow out into the embryo sac.

More recently SCHÜRHOFF[24] has investigated *Calycanthus fertilis*, *C. occidentalis*, and *C. floridus*, all of which show similarities in the development of their embryo sacs. He finds, in common with LONGO and PETERS, a many-celled hypodermal apical sporogenous mass of cells in each nucellus, whose cells without cutting off primary wall cells develop into embryo sac mother cells. There is formed from the dermatogen a many-celled nucellar cap over the micropylar ends of the embryo sacs. SCHÜRHOFF believes that no chromosome reduction occurs in the formation of the megaspore. During the first division of the nucleus of the megaspore both synapsis and diakinesis are described, but after spindle formation the "gemini" pass to the equatorial plate, where each undergoes a longitudinal split, thus resulting in a distribution of a diploid number of chromosomes to each daughter nucleus. Unfortunately, SCHÜRHOFF's figures and reproductions of reduction stages of nuclear division are very inadequate, and do not seem to the reviewer to permit the conclusions drawn from them. Fig. 1 is labeled "synapsis" in a pollen mother cell, but does not in any way resemble any stage of synapsis as found and described by the reviewer for *Calycanthus floridus*. According to SCHÜRHOFF the embryo arises apogamously from the unfertilized egg and not from nucellar tissue as stated by PETERS. The endosperm, which is cellular from the first, contains diploid nuclei and is developed from the lower polar nucleus, as observed by PETERS. In all the species studied by SCHÜRHOFF the haploid number of chromosomes is twelve.

The pollen mother cells form tetrads by the simultaneous formation of walls, although during tetrad formation disturbances in development occur. Many pollen grains degenerate during the two-nucleate stage, and those that mature do not germinate.—J. B. OVERTON.

Iron in nutrient solutions.—In ordinary nutrient solutions, the availability of the iron added depends partly upon the iron salts used, and partly upon the acidity of the culture medium. TOTTINGHAM and RANKIN[25] have tested the availability of four salts of iron: ferric citrate, phosphate, and sulphate, and ferrous sulphate, for the growth of wheat. Ferric phosphate is relatively

[23] PETERS, J., Zur Entwicklungsgeschichte einiger Calycanthaceae. Beitr. Biol. Pflanz **14**:1920.

[24] SCHÜRHOFF, P. N., Zur Apogamie von *Calycanthus*. Flora **116**:73–84. 1923.

[25] TOTTINGHAM, W. E., and RANKIN, E. J., The availability of iron in nutrient solutions for wheat. Amer. Jour. Bot. **10**:203–210. 1923.

insoluble over a considerable range of hydrogen ion concentration; and both ferric and ferrous sulphates are relatively insoluble in solutions nearly neutral in reaction. Ferric citrate is not much more soluble than the others, but remains soluble in about the same proportion over a wide range of hydrogen ion concentration. On the average, 60 per cent of the citrate goes into solution when 10 mg. per liter are supplied, and this salt provides enough iron for good growth, especially if the solutions are renewed from time to time.

Ferric sulphate increases hydrogen ion concentration with time, while ferric citrate has the opposite effect. Ferric phosphate and ferrous sulphate do not modify the reaction of the solution noticeably. The citrate gave the most favorable growth measurements, and it seems clear that the variation in efficiency of the iron salts used is related to solubility or the modification of the solution reaction.—C. A. SHULL.

Anion-cation relations in nutrient solutions.—In a brief paper, GERICKE[26] discusses the results of an experiment in which wheat seedlings were grown for one day periods in dilute solutions of single salts, and then transferred to corresponding solutions of other nutrient salts, the order being repeated over and over through the growing period. The order of change from one to another was of minor importance, but the kind of salts used in providing the six necessary ions was of great importance. Since only one salt was used at a time, the growth differences are ascribed to the way in which essential ions are paired. Thus nitrate was secured in proper amount only when it was grouped with potassium. The absence of any one of the other essential elements for forty-eight hours was not appreciably injurious. Seedlings held for twenty-four hours in $MgHPO_4$ were not harmed; but if $MgSO_4$ or $Mg(NO_3)_2$ were used, decided injury resulted. These injuries are not due to the variations in reaction value of the solutions used. The proper pairing of ions in the nutrient solution may be an important factor in physiological balance.—R. B. DUSTMAN.

Embryo of Lemna.—BLODGETT[27] has investigated the embryogeny of *Lemna perpusilla*, with the following results. The embryo develops without the formation of a suspensor, all the cells from the egg entering into the formation of the embryo. There is, therefore, no proembryo differentiated later into suspensor and embryo. The stem tip appears as a lateral mound of cells, and develops directly into the first leaf or plumule, which becomes inclosed by a cotyledon sheath. A "daughter frond" is soon formed in the larger basal lobe of the plumule, and this develops the first root structure. The plumule protrudes from the cotyledon sheath at germination in such a way as to lie upon the water surface. In the species studied, the author found that flowers are produced freely, and the presence of minute insects suggested the possibility of insect pollination.—J. M. C.

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[26] GERICKE, W. F., On the physiological balance in nutrient solutions for plant cultures. Amer. Jour. Bot. **9**:180–183. 1922.

[27] BLODGETT, F. H., The embryo of *Lemna*. Amer. Jour. Bot. **10**:336–342. *pl. 28. fig. 1.* 1923.